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(54) Elongated and multiple spacers containing activatible prodrugs

(57) This invention is directed to prodrugs that can be activated at the preferred site of action in order to selectively deliver the corresponding therapeutic parent drugs to target cells or to the target site. This invention will therefore primarily but not exclusively relate to tumor cells as target cells. More specifically the prodrugs are compounds of the formula $V\text{-}(W)_k\text{-}(X)_l\text{-}A\text{-}Z$, wherein:

V is a specifier; W and X are each a 1, (4+2n) electronic cascade spacer, being the same or different;
A is either a spacer group of formula $(Y)_m$, wherein:

Y is a 1, (4+2n) electronic cascade spacer, or a group of formula
U being a cyclisation elimination spacer;

Z is a therapeutic drug;
k, l and m are integers from 0 to 5;
n is an integer of 0 to 10,

with the provisos that:

- when A is $(Y)_m$: $k+l+m \geq 1$, and if $k+l+m=1$, then $n > 1$;
- when A is U: $k+l \geq 1$.

specifier —— (elongated spacer system)—— drug

FIG. 2

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Description

[0001] This invention is directed to prodrugs that can be activated at the preferred site of action in order to selectively deliver the corresponding therapeutic parent drugs to target cells or to the target site. This invention will therefore primarily but not exclusively relate to tumor cells as target cells.

[0002] Lack of selectivity of chemotherapeutic agents is a major problem in cancer treatment. Because highly toxic compounds are used in cancer chemotherapy, it is typically associated with severe side effects. Drug concentrations that would completely eradicate the tumor cannot be reached because of dose-limiting side effects such as gastrointestinal tract and bone marrow toxicity. In addition, tumors can develop resistance against anticancer agents after prolonged treatment. In modern drug development, targeting of cytotoxic drugs to the tumor site can be considered one of the primary goals.

[0003] A promising approach to obtain selectivity is to exploit the existence of tumor-associated enzymes. A high level of tumor-specific enzyme can activate a pharmacologically inactive prodrug to the corresponding active parent drug in the vicinity of the tumor. Via this concept a high concentration of toxic anticancer agent can be generated at the tumor site. All tumor cells may be killed if the dose is sufficiently high and consequently no drug resistant tumor cells can develop.

[0004] There exist several enzymes which are present at elevated levels in certain tumor tissues. One example is the enzyme β-glucuronidase, which is liberated from certain necrotic tumor areas. Furthermore, several proteolytic enzymes have been shown to be associated with tumor invasion and metastasis. Several proteases, like for example the cathepsins and proteases from the urokinase-type plasminogen activator (u-PA) system are all involved in tumor metastasis. The serine protease plasmin plays a key role in tumor invasion and metastasis. The proteolytically active form of plasmin is formed from its inactive pro-enzyme form plasminogen by u-PA. The tumor-associated presence of plasmin can be exploited for targeting of plasmin-cleavable prodrugs.

[0005] In this invention a new technology is disclosed that can be applied to prepare prodrugs or conjugates for targeting drugs to disease-related or organ-specific tissue or cells, for example tumor-specific prodrugs. This technology can furthermore find application in (nonspecific) controlled release of compounds, with the aim of facilitating release. The present invention is deemed to be applicable to all drugs that need to be delivered at a specific target site where a specific disease-related biomolecule can convert the prodrug into the drug or induce conversion of the prodrug into the drug.

[0006] The technology of this invention relates to novel linker systems to be inserted between specifier (= part of prodrug to be cleaved by the enzyme) and parent drug. A great number of anticancer prodrugs that have been developed in the past contain a self-immolative connector or linker, also called self-elimination spacer. This spacer is incorporated between the specifier and the drug in order to facilitate enzymatic cleavage and so enhance the kinetics of drug release (as shown in figure 1). The specifier (which for example can be an oligopeptide substrate for a protease or for example a β-glucuronide substrate for β-glucuronidase) must be site-specifically removed, followed by a spontaneous spacer elimination to release the cytotoxic parent drug. In this invention, greatly improved linker systems are disclosed. These are applicable in prodrugs, for example anticancer prodrugs, and significantly enhance enzymatic activation rates.

[0007] More specifically, the invention relates to compounds of the formula:

$$V\text{-}(W)_k\text{-}(X)_1\text{-}A\text{-}Z$$

wherein:

V is an enzymatically removable specifier,
W and X are each a 1,(4+2n) electronic cascade spacer, being the same or different,
A is either a spacer group of formula $(Y)_m$, wherein
Y is a 1, (4+2n) electronic cascade spacer, or a group of formula U, being a cyclisation elimination spacer,
Z is a therapeutic drug,
k, 1 and m are independently an integer of 0 (included) to 5 (included),
n is an integer of 0 (included) to 10 (included),

with the provisos that:

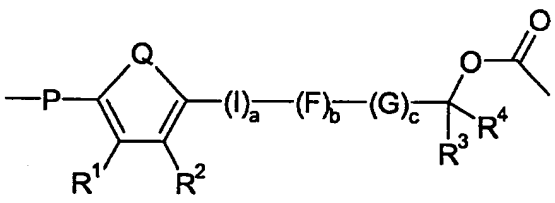
- when A is $(Y)_m$: then $k+1+m \geq 1$, and
  if $k+l+m=1$, then $n > 1$;
- when A is U: then $k+l \geq 1$.

[0008] These novel elongated linker systems show improved enzymatic activation characteristics, which is demonstrated in the following examples.

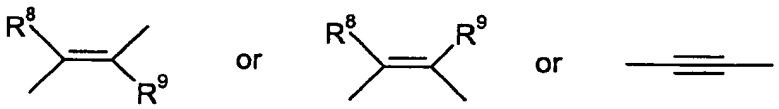
An activatible prodrug according to this invention comprises a specifier V, which is meant to consist of a group, which can be site specifically removed, and which is covalently attached to a therapeutic drug *via* the novel elongated self-immolative connector systems of the invention (figure 2). These self-immolative connector systems possess increased lengths, which places the parent drug at an increased distance from the specifier.

[0009] It is observed that spacers which eliminate through a 1, (4+2n)-elimination (n = 0,1,2,3,4,5...10) (for example 1,6-elimination, 1,8-elimination, or 1,10-elimination) are from now called 'electronic cascade' spacers.

[0010] According to a preferred embodiment of the invention are the electronic cascade spacers W, X and Y independently selected from compounds having the formula:



wherein Q = $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$ P = NR^7, O, S a, b, and c are independently an integer of 0 (included) to 5 (included); I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are idependently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

[0011] It is further observed that the principle of 1,6-elimination, as such developed in 1981, can be considered one of the most versatile self-elimination principles that can be used in prodrug design. In this principle, spacer elimination proceeds via the mechanism depicted in figure 3. This particular elimination process has proven to be very successful when applied in the prodrug concept. Spacers that self-eliminate through an electronic cascade sequence as indicated in figure 3 generally show much faster half-lives of elimination than do spacers that eliminate via a cyclisation reaction. This is a significant difference between cyclisation spacers and electronic cascade spacers.

In the following Examples, para-aminobenzyloxycarbonyl (PABC) electronic cascade spacer systems are used because they eliminate more rapidly upon unmasking of the amine. In contrast to aminobenzyl spacer systems, hydroxybenzyl electronic cascade spacers need electron-withdrawing substituents on the phenyl part of the spacer in order to let spacer elimination take place. Drug release will not take place when the spacer is an un-substituted hydroxybenzyl electronic cascade spacer.

Most efforts were, in the past, therefore directed to the synthesis of electronic cascade spacers containing electron-withdrawing substituent(s). It was hypothesized that the withdrawal of electrons from the site where enzymatic activation occurs would enhance the rate of enzymatic activation. However, the activation rate of prodrugs containing an electron-withdrawing group on the spacer is usually not significantly different from that of un-substituted electronic cascade spacer containing prodrugs: A chloro-substituent on an aminobenzyl spacer for example only marginally enhances the rate of enzymatic prodrug activation by plasmin. In the case of aminobenzyl spacer containing anthracycline prodrugs for activation by β-glucuronidase, chloro- or bromo-substituents showed only a marginal effect. It must further be considered that, although electron-withdrawing substituents on aminobenzyl spacers may increase enzymatic activation

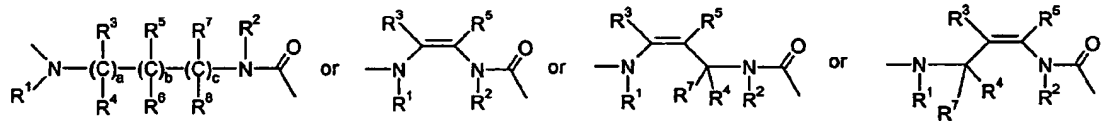
rates, spacer elimination rates will decrease as a consequence of substituents with such electronic properties. In the case of generation of hydroxylamino benzyl electronic cascade spacers it appeared that indeed electron-donating substituents on the benzyl ring accelerated fragmentation. This effect can probably be ascribed to stabilization of the developing positive charge on the benzylic carbon by these substituents. In some cases, when one or more of the spacer substituents are too electron-withdrawing, spacer elimination will not occur at all. An aminobenzyl spacer containing a nitro substituent at the meta position with respect to the specifier did not self-eliminate to release the free drug. It was also found that a hydroxylamino benzyl spacer with a nitro substituent at the meta position with respect to the specifier showed the slowest spacer elimination rate of such substituted hydroxylamino benzyl spacers. It appears that electron-withdrawing properties of spacer substituents have only marginal impact on enzymatic activation rates, whereas spacer elimination is greatly dependent on electronic properties of spacer substituents and occurs only in a relatively narrow characteristic electronic profile depending on the type of cascade spacer that is used.

[0012] In several known, one electronic cascade spacer containing prodrugs differences in enzymatic activation rates can still be observed when different parent drugs are connected with the same promoiety or when a parent drug is connected to the same promoiety via a different site of the drug. For example, β-glucuronidase cleaves the glucuronide from a β-glucuronide-cyclisation spacer promoiety much slower when paclitaxel is the parent drug in comparison with the prodrug containing doxorubicin as the parent drug. In another example, a dipeptide derivative of paclitaxel, linked via an aminobenzyl spacer was more readily cleaved by cathepsin B when paclitaxel was linked via its 7-position than via its 2'-position. In addition, half-lives of cathepsin B cleavage of electronic cascade spacer containing prodrugs of doxorubicin or mitomycin C were much shorter than the half-life of the corresponding prodrugs with paclitaxel as the parent drug. Finally, plasmin cleaves the tripeptide from an electronic cascade spacer containing doxorubicin prodrug much more readily than the tripeptide from the corresponding paclitaxel prodrug. Thus, in several prodrug systems the parent drug still exerts a significant effect on the rate of enzymatic activation, even though the mentioned prodrugs all contained one electronic cascade spacer.

[0013] The invention obviates the above-mentioned drawbacks by reduction of the influence of substituents of the spacer group on prodrug activation and/or spacer elimination and of the parent drug on the rate of enzymatic activation of the prodrug due to the presence of elongated spacer systems.

[0014] The invention is in a second aspect related to compounds of the above-mentioned formula wherein group U is a cyclisation spacer, from now called ω-amino aminocarbonyl'cyclisation spacer, and Z is a drug having a hydroxyl group.

[0015] More preferably, the ω-amino aminocarbonyl cyclisation elimination spacer U, of the invention is a compound having the formula:



wherein:

a is an integer of 0 or 1; and
b is an integer of 0 or 1; and
c is an integer of 0 or 1; provided that
a + b + c = 2 or 3;

and wherein R^1 and/or R^2 independently represent H, C_{1-6} alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x{}^1R_x{}^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, $R_x{}^1$ and $R_x{}^2$ are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7, and R^8 indepently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x{}^1R_x{}^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl (S

$(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonooxy (OP(=O) $(OH)_2$), and phosphate (OP(=O) $(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

[0016] The invention is further explained by the accompanying drawings, wherein:

Figure 1 shows schematically the conversion of a spacer containing tripartate prodrug into the parent drug.
Figure 2 shows schematically the structure of an elongated spacer containing prodrug.
Figure 3 shows the principle of 1,6-elimination.
Figure 4 shows the principle of 1,8-elimination.
Figure 5 shows the principle of 1,10-elimination.
Figure 6 shows the synthesis of the model paclitaxel-containing compound to prove the principle of 1,8-elimination.
Figure 7 shows the mechanism for the release of paclitaxel after reduction and 1,8-elimination.
Figure 8 shows the synthesis of the doubly Aloc-protected D-Ala-Phe-Lys tripeptide.
Figure 9 shows the synthesis of the 1,8-elimination spacer paraaminocinnamyl alcohol (PACA).
Figure 10 shows the synthesis of a 1,8-elimination spacer containing prodrug.
Figure 11 shows schematically the structure of a prodrug containing two or more electronic cascade spacers.
Figure 12 shows the synthesis of para-nitrophenyl (PNP) carbonate-activated tripeptide-spacer conjugate.
Figure 13 shows the catalytic coupling of a second electronic cascade spacer molecule (para-aminobenzyl alcohol (PABA)) to the 4-nitrophenyl carbonate-activated tripeptide-spacer conjugate in the presence of hydroxy benzotriazole (HOBt).
Figure 14 shows a reaction to chemically link two electronic cascade spacer molecules by coupling a second electronic cascade 1,6-elimination spacer molecule to a 4-nitrophenyl carbonate activated tripeptide-1,6-elimination spacer conjugate in the presence of catalytic amounts of diphenyl phosphinic acid.
Figure 15 shows the synthesis of a doxorubicin containing double 1,6-elimination spacer containing prodrug.
Figure 16 shows the synthesis of a paclitaxel containing double 1,6-elimination spacer containing prodrug.
Figure 17 shows the synthesis of a tryptophan-containing tripeptide double spacer conjugate.
Figure 18 shows the synthesis of a tryptophan-containing doxorubicin prodrug.
Figure 19 shows the synthesis of a doxorubicin containing triple 1,6-elimination spacer containing prodrug.
Figure 20 shows schematically the structure of a prodrug of paclitaxel that contains both an electronic cascade spacer and a cyclisation spacer coupled to the drug via a 2'-carbamate linkage.
Figure 21 shows the regioselective synthesis of 2'-(4-nitrophenyl carbonate) activated paclitaxel using 4-nitrophenyl chloroformate at low temperature.
Figure 22 shows the synthesis of the acid protected paclitaxel-ω-amino aminocarbonyl cyclisation spacer conjugate.
Figure 23 shows the coupling of the acid protected paclitaxel-ω-amino aminocarbonyl cyclisation spacer conjugate to the 4-nitrophenyl carbonate activated tripeptide-1,6-elimination spacer conjugate.
Figure 24 shows the deprotection reaction to obtain the paclitaxel prodrug that contains a 1,6-elimination spacer and an ω-amino aminocarbonyl cyclisation spacer.
Figure 25 shows the structure of previously reported doxorubicin and paclitaxel prodrugs containing one electronic cascade spacer.

[0017] In the compounds of formula V-$(W)_k$-$(X)_1$-A-Z, the specifier V is typically a substrate molecule that is specifically cleaved by an enzyme present in the vicinity of the target cells, for example tumor cells. In one embodiment, the specifier is an oligopeptide which consists of an amino acid sequence specifically recognized by a protease present in the vicinity of the target cells, for example tumor cells, or, in another embodiment, a β-glucuronide that is specifically recognized by β-glucuronidase present in the vicinity of tumor cells. In again another embodiment the specifier is a nitro-aromatic moiety that can be reduced under hypoxic conditions or by nitroreductases. After removal of the nitro-aromatic specifier, elimination of the spacer systems described in this invention leads to drug release. It can be understood that any specifier that is specifically cleaved following recognition by a disease-specific and/or organ-specific enzyme and/or receptor can be incorporated into prodrugs that contain the linker systems claimed in this invention.

[0018] In one embodiment the elongated spacer is a molecule that self-eliminates via a 1, (4+2n)-elimination (n = 2,3,4,5...10), for example a 1,8-elimination (figure 4). The length of this novel spacer system can be extended, for example to a 1,10-elimination system, in which two or more double or triple bonds instead of one are conjugated with the aromatic part of the spacer (figure 5).

In another embodiment, the spacer system of the invention consists of two or more electronic cascade spacers that are connected to one another. Release of the leaving group (the drug) occurs after two or more subsequent spacer

eliminations. The elongated spacer systems provide for improved enzymatic activation characteristics.

[0019] In again another embodiment, prodrugs of hydroxyl functionality containing drugs (such as for example paclitaxel) are claimed that contain both one or more electronic cascade spacers and a cyclisation spacer. In a preferred embodiment the spacer that is directly connected to the paclitaxel molecule is an ω-amino aminocarbonyl cyclisation spacer that is linked to the 2'-position of paclitaxel via a carbamate linkage. A convenient synthetic route to this class of paclitaxel derivatives is disclosed.

[0020] The self-immolative connector systems in this invention possess increased lengths with respect to an electronic cascade spacer system available at present. It is observed that one end of the linker system must be able to react with the specifier, for example the tripeptide that is a substrate for plasmin. Typically, this end of the spacer system is an amino group or a hydroxyl group, but it can also be another functionality. The functionality at the other end of the linker system must be able to react with the drug. Typically, this end of the spacer system is a hydroxyl group, but it can also be another functionality. In one embodiment this functionality reacts with an amino group of the drug to form a carbamate linkage between linker and drug. In another embodiment, this functionality reacts with a hydroxyl group of the drug to form a carbonate linkage between linker and drug. In again another embodiment, this functionality reacts with a sulfhydryl group of the drug to form a thiocarbonate linkage between linker and drug. In again another embodiment this functionality reacts with a carboxylic acid group of the drug to form an ester linkage between linker and drug.

[0021] Typically, the therapeutic drug is an anticancer drug. Preferably the anticancer drug is the amino containing daunorubicin, doxorubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, mitomycin C, mitomycin A, 9-amino camptothecin, aminopterin, actinomycin, bleomycin, N^8-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, tallysomycin, or derivatives thereof. The drug can also be the hydroxyl containing etoposide, camptothecin, irinotecan, topotecan, 9-amino camptothecin, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, doxorubicin, morpholine-doxorubicin, N-(5,5-diacetoxypentyl) doxorubicin, vincristine, vinblastine, or derivatives thereof. The drug can also be the sulfhydryl containing esperamicin, 6-mercaptopurine, or derivatives thereof. The drug can also be the carboxyl containing methotrexate, camptothecin (ring-opened form of the lactone), butyric acid, retinoic acid, or derivatives thereof.

[0022] To show the principle of elimination of elongated spacer systems, tumor-specific prodrugs that are selectively hydrolyzed by the tumor-associated protease plasmin, were synthesized. The synthesized prodrugs consisted of a tripeptide specifier that was coupled to the drug via an elongated self-eliminating spacer. The tripeptide specifier contains an amino acid sequence that is specifically recognized by the tumor-associated enzyme plasmin. The synthesis of these derivatives is disclosed.

[0023] There is an increasing body of literature that links production of certain proteases to tumor malignancy. Mostly, proteolytic activity is required for tumor cells when they become invasive and form metastases. A primary tumor is encapsulated in an extracellular matrix, which consists of proteins. In order to form metastases, the primary tumor must break through this matrix. For this reason, enhanced expression of proteolytic enzymes by invading and metastasizing tumors is generated. Recent studies indicate that proteases are involved also in earlier stages of tumor progression, at both primary and metastatic sites. A number of proteases, like cathepsins, the u-PA system, and the matrix metalloproteinases, take part in the proteolytic cascade.

The u-PA system has received broad attention in the literature, especially in the last decade. Several invasive and metastasizing human tumors express a significantly higher plasminogen activator activity in comparison with normal tissue. An increased activity and expression of u-PA is found in several tumor cell lines and human solid tumors, like lung tumors, prostate cancers, breast cancers, ovarian carcinomas and several other cancer types. u-PA is an important enzyme in proteolytic reactions that are required for the spreading and invasiveness of cells, both in cancer and in tissue remodeling processes. u-PA interacts with a specific high-affinity receptor on the cell surface. Receptor-bound u-PA is catalytically active on the surface of the cell without requiring internalization. It interacts with plasminogen to produce plasmin that is still bound to the cell surface. The high u-PA level via this pathway leads to elevated levels of plasmin. There exists substantial evidence that the protease plasmin itself plays a key role in tumor invasion and metastasis. Plasmin itself catalyses the breakdown of extracellular matrix proteins. Thus, the plasminogen activator system is intimately associated with tumor metastasis. Even the process of angiogenesis, nowadays considered as an important target mechanism for the development of new therapeutic strategies, is a urokinase dependent process. The plasminogen activation system may be involved in cell adhesion processes by regulating integrin functions. Vascular endothelial growth factor (VEGF), an angiogenic molecule, is suggested to interact with u-PA in tumor progression. u-PA catalyzed plasmin generation proved to be an important determinant of tumor metastasis in many experiments with animal model systems.

For the reasons outlined above, plasmin can be a very promising enzyme for the targeting of peptide prodrugs of anticancer agents. Active plasmin is localized in tumor tissue because it is formed from its inactive pro-enzyme form plasminogen by u-PA, produced by cancer and/or stroma cells. In the blood circulation active plasmin is very rapidly inhibited by inhibitors that block the active site, such as α_2-antiplasmin. Cell-bound plasmin as present in tumor tissue is not inhibited. In addition, plasmin is very suitable as a target enzyme for prodrugs, because it is generated at the

end of the proteolytic cascade. One molecule of u-PA can generate more than one molecule of plasmin.

[0024] The amino acid sequence of the tripeptide to be a plasmin substrate must be chosen such that it is a very specific substrate for the serine protease plasmin. The C-terminal amino acid that is coupled to the spacer-drug moiety is preferably an *L*-Lysine residue. Plasmin is known to cleave most easily after a Lysine residue. The amino acid at the N-terminus possesses the *D*-configuration in order to prevent *in vivo* cleavage by ubiquitous amino peptidases. Protecting the N-terminal amino function by a Boc or Fmoc group can also prevent unwanted peptidase cleavage. The amino acid in the middle is preferably a hydrophobic *L*-amino acid, e.g., *L*-Leucine or *L*-Phenylalanine. By converting the two amino groups of the tripeptide into the corresponding ammonium salts, the water solubility of the prodrug should be further improved.

[0025] In the present invention the synthesis and application of new elongated spacer systems is described. In one embodiment, this spacer self-eliminates through a 1,(4+2n)-elimination process (figures 4,5). These 1,(4+2n)-elimination spacers are elongated with respect to the conventional 1,6-elimination spacer. Proof of principle of 1,8-elimination was delivered upon chemical reduction of the nitrocinnamyl carbonate derivative of paclitaxel using Zn and acetic acid (figures 6,7). Released paclitaxel was isolated in good yield. Firstly, the doubly protected tripeptide was synthesized (figure 8). The 1,8-elimination spacer itself was synthesized from 4-nitrocinnamyl alcohol as depicted in figure 9. 4-Aminocinnamyl alcohol was incorporated between doxorubicin and a tripeptide for plasmin activation (figure 10).

What is also disclosed in this invention is the synthesis of prodrugs that contain two or more electronic cascade spacers connected to one another, incorporated between specifier and drug (figure 11). Prodrugs containing linker systems of this kind have not been reported before. This embodiment of the present invention was exemplified by synthesizing two prodrugs containing a tripeptide specifier coupled to doxorubicin or paclitaxel via two 1,6-elimination spacers. This protected tripeptide was subsequently coupled with 4-aminobenzyl alcohol, and the resulting benzylic alcohol was activated with 4-nitrophenyl chloroformate to yield the corresponding 4-nitrophenyl carbonate (figure 12). In a very efficient reaction a second molecule of 4-aminobenzyl alcohol was coupled to the activated carbonate in which hydroxy benzotriazole (HOBt) was employed as a catalyst to yield the corresponding tripeptide-double spacer conjugate (figure 13). When this reaction was performed using diphenyl phosphinic acid as a catalyst, the product was isolated in only 16 percent yield (figure 14). The peptide-double spacer conjugate was incorporated into a doxorubicin prodrug (figure 15) and a paclitaxel prodrug (figure 16), by subsequent chloroformate activation, coupling with the drug and final deprotection. A double spacer-containing doxorubicin prodrug with a tryptophan residue instead of phenylalanine was also synthesized (figures 17,18). According to a further embodiment a third 4-aminobenzyl alcohol spacer was reacted with the 4-nitrophenyl carbonate activated tripeptide-double spacer conjugate to yield the corresponding tripeptide-triple spacer conjugate (figure 19). This compound was subsequently converted to the corresponding triple electronic cascade spacer containing doxorubicin prodrug employing a similar route as depicted in figures 15 and 16.

What is also claimed is a 2'-carbamate coupled paclitaxel prodrug with an elongated linker system that contains both one or more electronic cascade spacers and an ω-amino aminocarbonyl cyclisation spacer (figure 20). Complex prodrugs of this type were synthesized via a novel convergent route, which leads to high yields, as claimed in claim 12. Paclitaxel will be released after one or more 1, (4+2n)-eliminations (n = 0,1,2,3,4,5,....10) and a subsequent intramolecular cyclisation. In the present invention the cyclisation spacer is connected to the 2'-OH group of paclitaxel through a carbamate linkage (figure 20). Firstly, paclitaxel was selectively activated at the 2'-position (figure 21) Secondly, a mono-protected cyclisation spacer was coupled to the 2'-activated paclitaxel analog and the protective group was removed under acidic conditions to yield the first fragment (figure 22). The second fragment was synthesized by connecting the 1,6-elimination spacer to the tripeptide specifier and subsequent 4-nitrophenyl chloroformate activation of the benzylic alcohol function (figure 12). Then, both fragments were coupled to one another (figure 23) and the coupled product was deprotected (figure 24). Coupling of two separate fragments according to this strategy in which in the final stage the chemical link between the two spacers is established, did provide the most efficient route to the paclitaxel prodrug. This is a novel route to obtain prodrugs of this type, in which a specifier is connected to a hydroxyl containing drug via an electronic cascade spacer system (connected to the specifier) and a cyclisation spacer (connected to the drug). In figure 25 previously reported plasmin-activatible prodrugs containing one electronic cascade spacer are depicted.

[0026] The invention also relates to compounds as defined above, wherein the specifier V is removed by an enzyme that is transported to the vicinity of target cells or target tissue via ADEPT, PDEPT, VDEPT or GDEPT.

[0027] The invention is further exemplified by the following Examples. These examples are for illustrative purposes and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Synthesis of 2'-[4-nitrocinnamyl carbonate]-paclitaxel **1**.

[0028] To a solution of 200 mg (1.12 mmol, 4.8 equiv) 4-nitrocinnamyl alcohol in dry dichloromethane/tetrahydrofuran under an Argon atmosphere was added pyridine (94 µl, 5.0 equiv) and 4-nitrophenyl chloroformate (236 mg, 5.0 equiv). The reaction mixture was stirred for 12 h at room temperature. The mixture was cooled to 0 °C and a catalytic amount of DMAP, a few drops of triethyl amine and 200 mg paclitaxel (1.0 equiv) were added. The reaction mixture was stirred at room temperature for 12 h. Solvents were evaporated and the remaining solid was dissolved in dichloromethane. The organic layer was thoroughly washed with a saturated sodium bicarbonate solution, 0.5 N potassium bisulfate and brine and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow oil was purified by means of column chromatography (ethyl acetate-hexane; 1:1), to yield 144 mg of **1** (58%). M.P. 151 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.17 (s, 3H, 17), 1.22 (s, 3H, 16), 1.70 (s, 3H, 19), 1.96 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.46 (s, 3H, 4-OAc), 2.55 (m, 1H, 6a), 3.82 (d, 1H, J=7.0 Hz, 3), 4.26 (d, 1H, J=8.4 Hz, 20b), 4.32 (d, 1H, J=8.4 Hz, 20a), 4.39 (m, 1H, 7), 4.87 (bt, 2H, CH_2-spacer), 4.99 (bd, 1H, J=7.9 Hz, 5), 5.46 (d, 1H, J=2.8 Hz, 2'), 5.72 (d, 1H, J=7.1 Hz, 2), 6.01 (m, 1H, 3'), 6.26 (bt, 1H, 13), 6.34 (s, 1H, 10), 6.43 (dt, 1H, J=16.0 Hz, HC=CH-CH_2), 6.75 (d, 1H, J=16.0 Hz, HC=CH-CH_2), 7.35-7.67 (m, 13H, aromatic), 7.75 (d, 2H, J=7.2 Hz, aromatic), 8.15 (d, 2H, J=7.2 Hz, aromatic), 8.19 (d, 2H, J=8.7 Hz, nitrophenyl) ppm; MS (FAB) *m/e* 1059 $(M + H)^+$, 1081 $(M + Na)^+$; Anal. ($C_{57}H_{58}N_2O_{18}\cdot 2½H_2O$) calculated C 62.01%, H 5.75%, N 2.54%, measured C 62.06%, H 5.31%, N 2.60%.

Example 2

Principle of 1,8-elimination: chemical reduction of the nitrocinnamyl carbonate **1**.

[0029] 36 mg of 2'-[4-nitrocinnamyl carbonate]-paclitaxel **1** was dissolved in 8 ml methanol and 2 ml acetic acid. A catalytic amount of Zinc powder was added and the red mixture was stirred for 12 h. Dichloromethane was added and the organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate, brine, and water and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (ethyl acetate - hexane; 2:1), to yield 28 mg of paclitaxel (confirmation by 300 MHz ^{1}H-NMR) and 4 mg of unreacted starting compound. When the compound was stirred in the absence of zinc powder under the same conditions, no paclitaxel was formed, indicating that reduction of the nitro group by zinc leads to the release of paclitaxel.

Example 3

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-OH **9**

Step a: Synthesis of Fmoc-Phe-Lys(Boc)-OBu **4**

[0030] To a solution of 2.50 g Fmoc-Phe-ONSu **2** (ONSu = N-hydroxysuccinimide) (5.16 mmol) in dry dichloromethane under an Argon atmosphere were added at 0 °C 0.791 ml triethyl amine (1.1 eq.) and 1.92 g H-Lys(Boc)-OBu·HCl **3** (1.1 eq.). The reaction mixture was stirred at room temperature for 5 hours, then dichloromethane was added and the organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulphate and evaporated. The resulting white solid **4** (3.08 g, 89%) was used without further purification. M.P. 93 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.10-1.90 (m, 24H, 6 CH_2-Lys and 18 tert-butyl), 3.06 (m, 2H, N-CH_2-Lys and benzylic), 4.19 (t, 1H, Fmoc), 4.25-4.55 (m, 4H, 2 Fmoc and 2 Hα), 7.19-7.78 (m, 13H, aromatic) ppm; MS (FAB) *m/e* 672 $(M + H)^+$, 694 $(M + Na)^+$; $C_{39}H_{49}N_3O_7$ calculated C 69.72%, H 7.35%, N 6.25%, measured C 69.69%, H 7.48%, N 6.22%.

Step b: Synthesis of Boc-D-Ala-Phe-Lys(Boc)-OBu **7**

[0031] 3.08 g (4.58 mmol) of Fmoc-Phe-Lys(Boc)-OBu **4** was dissolved in 100 ml of dioxane/methanol/2N sodium hydroxide (70/25/5) and stirred at room temperature for approximately 1 hour. The reaction mixture was neutralised with acetic acid (0.571 ml) and organic solvents were evaporated. Water and dioxane was added and the solution was freeze dried. Diisopropylether was added to the resulting solid. After filtration, the filtrate was evaporated. The residual product 5 was dissolved in dry dichloromethane and added at 0 °C to a solution of 1.19 g (4.16 mmol) Boc-D-Ala-ONSu

6 and 0.634 ml (1.1 eq.) of triethyl amine in dry dichloromethane. The reaction mixture was stirred overnight after which dichloromethane was added. The organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulphate and evaporated. The product was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH 20/1) to afford 2.56 g (4.13 mmol, 99%) of Boc-D-Ala-Phe-Lys(Boc)-OBu **7** as a white foam. M.P. 59 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.25 (d, 3H, CH_3-Ala), 1.43 (bs, 27H, tert-butyl), 1.00-1.90 (m, 6H, CH_2-Lys), 2.80-3.30 (m, 4H, N-CH_2-Lys and benzylic), 4.15 (m, 1H, Hα), 4.35 (m, 1H, Hα), 4.64 (bd, 1H, Hα), 7.15-7.35 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 621 $(M + H)^+$, 643 $(M + Na)^+$; $C_{32}H_{52}N_4O_8$ (·½ H_2O) calculated C 61.03%, H 8.48%, N 8.90%, measured C 61.15%, H 8.44%, N 8.66%.

Step c: Synthesis of D-Ala-Phe-Lys-OH **8.**

[0032] 2.56 g (4.13 mmol) Boc-D-Ala-Phe-Lys(Boc)-OBu **7** was stirred in a solution of HCl in EtOAc (3M). After 5 hours the solvent was evaporated, tert-butanol was added and evaporated twice to remove remaining hydrochloric acid. The resulting product was freeze dried in a mixture of dioxane/water to yield a cream coloured powder **8,** which was used without further purification. ^{1}H-NMR (300 MHz, D_2O): δ 0.94 (d, 3H, CH_3-Ala), 1.10-1.85 (m, 6H, CH_2-Lys), 2.75-2.84 (m, 3H, N-CH_2-Lys and benzylic), 3.09 (dd, 1H, benzylic), 3.54 (dd, 1H, Hα), 3.98 (dd, 1H, Hα), 4.54 (q, 1H, Hα), 7.10-7.22 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 365 $(M + H)^+$.

Step d: Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-OH **9.**

[0033] To solution of 706 mg (1.61 mmol) D-Ala-Phe-Lys-OH **8** in water/acetonitrile was added triethyl amine until a pH of 9 - 9.5 was reached. Then a solution of 704 mg (2.2 eq.) Aloc-ONSu in acetonitrile was added and the reaction mixture was kept basic by adding triethyl amine. After the pH of the mixture did not alter anymore, a 0.5 M solution of HCl was added until a pH of 3 was reached. The mixture was thoroughly extracted with dichloromethane. The organic layer was washed with water and the water layer was extracted again with dichloromethane. The organic layer was dried over anhydrous sodium sulphate and evaporated to dryness to result in the desired product **9** as a cream coloured foam (742 mg, 86%). M.P. 141 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.10-1.95 (m, 6H, CH_2-Lys), 1.21 (d, 3H, CH_3-Ala), 2.90-3.30 (m, 4H, N-CH_2-Lys and benzylic), 4.20 (m, 1H, Hα), 4.55 (m, 5H, Hα and 4 Aloc), 4.76 (bd, 1H, Hα), 5.17-5.31 (m, 4H, Aloc), 5.83-5.92 (m, 2H, Aloc), 7.20-7.28 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 533 $(M + H)^+$, 555 $(M + Na)^+$; $C_{26}H_{36}N_4O_8$ calculated C 58.63%, H 6.81%, N 10.52%, measured C 58.54%, H 6.81%, N 10.28%.

Example 4

Synthesis of 4-aminocinnamyl alcohol **10.**

[0034] To a solution of 489 mg (2.73 mmol) of 4-nitrocinnamyl alcohol in methanol/acetic acid was added a catalytic amount of activated zinc powder. The mixture was stirred at room temperature for 2.5 h. Then the reaction mixture was evaporated and dichloromethane was added. The organic layer was washed with aqeous sodium hydroxide (1M) and brine, dried over sodium sulfate, and evaporated yielding a red solid. The product was purified by means of column chromatography (ethyl acetate-heptane; 2:1 with 1% triethyl amine) to afford 98 mg (24%) of 4-aminocinnamyl alcohol **10:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24 (d, 2H, J = 6.1 Hz, CH_2OH), 6.11-6.20 (dt, 1H, J = 6.1 Hz, J = 15.8 Hz, CH=CH-CH_2OH), 6.48 (d, 1H, J = 15.8 Hz, benzylic), 6.62 (d, 2H, J = 11.1 Hz, aromatic), 7.19 (d, 2H, J = 11.0 Hz, aromatic) ppm; MS (EI) *m/e* 149$(M)^+$.

Example 5

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PACA **11.**

[0035] A solution of 148 mg (0.278 mmol) Aloc-D-Ala-Phe-Lys(Aloc)-OH **9** was dissolved in dry THF under an argon atmosphere and cooled to -40°C.

[0036] N-methyl morfoline (35 µL, 1.1 equiv) and isobutyl chloroformate (41 µL, 1.1 equiv) were added. The reaction mixture was stirred for 3 h at a temperature below -30 °C. A solution of 4-aminocinnamyl alcohol **10** (52 mg, 1.2 equiv) and N-methyl morfoline (38 µL, 1.2 equiv) in dry THF was added dropwise to the reaction mixture at a temperature of -50 °C. After seven days, THF was evaporated and dichloromethane was added. The organic layer was washed with saturated sodium bicarbonate, a 0.5 N potassium bisulfate solution, and brine, dried over sodium sulfate, and evaporated. The residual red solid was purified by means of column chromatography (chloroform- methanol; 15:1) to afford 124 mg (67%) of the desired product **11.** ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 1.23 (d, 3H, J = 7.0 Hz, CH_3-Ala), 1.25-2.05 (m, 6H, 3 CH_2-Lys), 2.75-3.28 (m, 4H, benzylic Phe and N-CH_2-Lys), 3.95-4.60 (m, 7H, 3Hα and 4H Aloc),

4.26 (d, 2H, J = 5.6 Hz, CH_2OH), 5.17-5.32 (m, 4H, Aloc), 5.68-5.75 (m, 1H, Aloc), 5.75 -5.95 (m, 1H, Aloc), 6.23-6.31 (dt, 1H, J = 15.9 Hz, J = 5.6 Hz, CH=CH-CH_2OH), 6.56 (d, 1H, J = 15.9 Hz, benzylic), 7.14-7.28 (m, 5H, aromatic Phe), 7.33 (d, 2H, J = 8.6 Hz, aromatic), 7.56 (d, 2H, J = 8.2 Hz, aromatic) ppm; MS (FAB) *m/e* 686 $(M + Na)^+$.

Example 6

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PACA-PNP **12.**

[0037] To a solution of 41.9 mg (0.0631 mmol) Aloc-D-Ala-Phe-Lys(Aloc)-PACA **11** in dry DMF containing 4Å molecular sieves under an argon atmosphere, bis(4-nitrophenyl)carbonate (58 mg, 3.0 eq.) and dry DIPEA (34µl, 3.0 eq.) were added. After stirring the mixture for 48 h, the mixture was evaporated to dryness. Then, chloroform was added and the organic layer was washed with 10% citric acid, brine and water, dried (Na_2SO_4) and evaporated yielding a yellow solid. The product was purified by means of column chromatography (chloroform-methanol; 9:1) to afford 33 mg (63%) of the carbonate **12.** ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.31 (d, 3H, J = 6.6 Hz, CH_3-Ala), 0.99-1.95 (m, 6H, CH_2-Lys), 2.80-3.55 (m, 4H, benzylic Phe and N-CH_2-Lys), 3.96-4.22 (m, 3H, Hα), 4.23-4.82 (m, 4H, Aloc), 4.98-5.12 (m, 2H, CH_2 spacer), 5.13-5.40 (m, 4H, Aloc), 5.48-5.99 (m, 2H, Aloc), 6.17-6.34 (m, 1H, CH=CH-CH_2 spacer), 6.74 (d, 1H, CH=CH-CH_2 spacer), 7.15-7.42 (m, 7H, aromatic Phe and spacer), 7.37 (d, 2H, J = 7.8 Hz, aromatic), 7.70 (d, 2H, aromatic), 8.28 (d, 2H, J = 8.0 Hz, aromatic) ppm.

Example 7

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PACA-DOX **13.**

[0038] Aloc-D-Ala-Phe-Lys(Aloc)-PACA-PNP **12** (40 mg, 0.0483 mmol) and doxorubicin-HCl (30 mg, 1.1 eq.) in N-methylpyrrolidinone were treated at room temperature with triethylamine (7.2 µl, 1.1 eq.). The reaction mixture was stirred in the dark for 16 h and was then diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with brine and water, and was dried (Na_2SO_4). After evaporation of the solvents the crude product was purified by means column chromatography (chloroform-methanol; 9:1) and by means of circular chromatography using a chromatotron supplied with a 2 mm silica plate (chloroform-methanol; 9:1), to yield 25 mg (42%) of protected prodrug **13.** ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.12-1.52 (m, 6H, sugar CH_3 and CH_3-Ala), 1.53-2.08 (m, 8H, CH_2-Lys and 2'), 2.15 (br d, 1H, J = 15.8 Hz, 8), 2.29 (br d, 1H, J = 15.4 Hz, 8), 2.82 - 3.39 (m, 6H, benzylic Phe and N-CH_2-Lys and 10), 3.68 (s, 1H, 4'), 3.86 (br d, 1H, 3'), 4.04 (br s, 4H, OMe and 5'), 4.05-4.30 (m, 2H, H α), 4.31-4.68 (m, 5H, Hα and 4 Aloc), 4.77 (s, 2H, 14), 5.03-5.22 (m, 2H, Aloc), 5.24 (br d, 2H, J = 10.5Hz, CH_2 spacer), 5.42-6.00 (m, 4H, 2H Aloc, 1' and 7), 6.03-6.18 (m, 1H, CH=CH-CH_2 spacer), 6.81 (d, 1H, J = 8.4 Hz, CH=CH-CH_2 spacer), 7.12 (d, 2H, J = 8.5 Hz, aromatic), 7.15-7.32 (m, 5H, aromatic Phe), 7.36 (d, 1H, J = 8.5 Hz, 3), 7.54 (d, 2H, J = 7.6Hz, aromatic), 7.75 (t, 1H, 2), 8.00 (d, 1H, J = 7.8 Hz, 1) ppm.

Example 8

Synthesis of D-Ala-Phe-Lys-PACA-DOX (·2HCl) **14.**

[0039] To a solution of 25 mg (0.0203 mmol) Aloc-D-Ala-Phe-Lys(Aloc)-PACA-DOX **13** in dry THF/dichloromethane under an argon atmosphere was added morpholine (17.8 µl, 10 eq.). Argon was bubbled through the reaction mixture for 15 minutes, after which a catalytic amount of $Pd(PPh_3)_4$ was added. The reaction mixture was stirred for 30 minutes in the dark, after which a second portion of catalytic $Pd(PPh_3)_4$ was added. Stirring (in the dark) was continued for 30 minutes. After addition of ice cold diethyl ether, the mixture was centrifuged and the red precipitate was collected after several washings with cold ether. A mixture of dioxane/water was added, the mixture was acidified with 3.8 ml of 12.5 mM hydrochloric acid, and subsequently freeze dried yielding 21.3 mg (92%) of doxorubicin prodrug **14.** ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.02-2.11 (m, 14H, sugar CH_3, CH_3-Ala, CH_2-Lys and 2'), 2.20 (br d, 1H, 8), 2.38 (br d, 1H, 8), 2.83-3.32 (m, 6H, benzylic Phe, N-CH_2-Lys and 10), 3.58-3.82 (m, 1H, 4'), 3.97 (s, 1H, 3'), 4.09 (s, 3H, OMe), 4.18 (m, 1H, 5'), 4.32-4.70 (m, 3H, Hα), 4.77 (s, 2H, 14), 5.28 (br d, 2H, CH_2 spacer), 5.40-5.62 (m, 2H, 1' and 7), 6.23 (m, 1H, CH=CH-CH_2 spacer), 6.82 (d, 1H, CH=CH-CH_2 spacer), 7.27 (d, 1H, J = 6.6 Hz, aromatic), 7.31-7.52 (m, 7H, aromatic Phe and spacer), 7.61 (d, 1H, J = 8.1 Hz, 3), 7.84 (t, 1H, 2), 8.05 (d, 1H, J = 7.6 Hz, 1) ppm.

Example 9

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABA **15.**

[0040] A solution of 730 mg (1.37 mmol) protected tripeptide Aloc-D-Ala-Phe-Lys(Aloc)-OH **9** was dissolved in dry THF under an Argon atmosphere and cooled to -40 °C. NMM (166 µl, 1.1 eq.) and isobutyl chloroformate (196 µl, 1.1 eq.) were added. The reaction mixture was stirred for 3 hours at a temperature below -30 °C. A solution of 4-aminobenzyl alcohol (203 mg, 1.2 eq.) and NMM (181 µl, 1.2 eq.) in dry THF was added dropwise to the reaction mixture. After 2 hours THF was evaporated and dichloromethane was added. The organic layer was washed with saturated sodium bicarbonate, a 0.5 N potassium bisulphate solution and brine, dried over anhydrous sodium sulphate, and evaporated. The residual pale yellow solid was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH 9/1) to afford 812 mg (93%) of the desired product **15** as a cream coloured powder. M.P. 156 °C; ^{1}H-NMR (300 MHz, DMSO-D^6) : δ 0.96 (d, 3H, CH_3-Ala), 1.10-1.85 (m, 6H, CH_2-Lys), 2.77 (dd, 1H, benzylic Phe), 2.97 (bd, 2H, N-CH_2-Lys), 3.09 (dd, 1H, benzylic Phe), 4.00 (t, 1H, Hα), 4.20-4.60 (m, 8H, 2 H α and 4 Aloc and CH_2-OH), 5.00-5.35 (m, 4H, Aloc), 5.76-5.95 (m, 2H, Aloc), 7.05-7.30 (m, 7H, aromatic), 7.41 (d, 1H, NH), 7.56 (d, 2H, aromatic), 8.12 (d, 1H, NH), 8.18 (d, 1H, NH), 9.80 (s 1H, NH anilide) ppm; MS (FAB) *m/e* 638 (M + H)$^+$, 660 (M + Na)$^+$; $C_{33}H_{43}N_5O_8$ (·½ H_2O) calculated C 61.29%, H 6.86%, N 10.83%, measured C 61.39%, H 6.54%, N 10.55%.

Example 10

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PNP **16.**

[0041] To a solution of 384 mg (0.602 mmol) **15** in dry THF/CH_2Cl_2 under an Argon atmosphere, 4-nitrophenyl chloroformate (182 mg, 1.5 eq.) and dry pyridine (73 µl, 1.5 eq.) were added. The reaction mixture was stirred at room temperature for 48 hours, then EtOAc was added. The organic layer was washed with 10% citric acid, brine and water, dried over anhydrous sodium sulphate and evaporated yielding a yellow solid. The product was purified by means of column chromatography (SiO_2 - CH_2Cl_2/MeOH 30/1) to afford 324 mg (67%) of carbonate **16.** ^{1}H-NMR (300 MHz, $CDCl_3$/CD_3OD) : δ 1.21 (d, 3H, CH_3-Ala), 1.25-2.05 (m, 6H, CH_2-Lys), 2.95 (dd, 1H, benzylic Phe), 3.13 (bt, 2H, N-CH_2-Lys), 3.27 (dd, 1H, benzylic Phe), 4.08 (dd, 1H, Hα), 4.25 (dd, 1H, Hα), 4.30-4.65 (m, 5H, Hα and 4 Aloc), 5.04-5.35 (m, 4H, Aloc), 5.26 (s, 2H, CH_2-OH), 5.65-6.00 (m, 2H, Aloc), 7.10-7.35 (m, 5H, aromatic), 7.39-7.43 (2 * d, 4H, aromatic), 7.71 (d, 2H, aromatic), 8.28 (d, 2H, aromatic) ppm; MS (FAB) *m/e* 803 (M + H)$^+$, 825 (M + Na)$^+$.

Example 11

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABA **17.**

[0042] To a solution of 156 mg (194 µmol) of compound **16** and 26.3 mg (1.1 eq.) PABA in dry N,N-dimethyl formamide under an Argon atmosphere was added diisopropylethyl amine (34 µl, 1.0 eq.) and a catalytic amount of N-hydroxybenzotriazole (7.9 mg, 0.3 eq.). The reaction solution was stirred for 24 hours after which it was diluted with 10% propanol-2/EtOAc. The organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium hydrogensulfate and brine, dried over anhydrous sodium sulfate and evaporated to dryness. The yellow residual film was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH 9/1) to yield 148 mg (97%) of the desired product **17**. M.P. 196-197°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.20 (d, 3H, ^{3}J=6.4 Hz, CH_3-Ala), 1.27-2.05 (m, 6H, 3 CH_2-Lys), 2.99 (dd, 1H, benzylic), 3.14 (m, 2H, N-CH_2-Lys), 3.27 (dd, 1H, benzylic), 4.00-4.64 (m, 7H, 3 Hα and Aloc), 4.57 (s, 2H, benzylic-spacer), 5.14 (s, 2H, benzylic-spacer), 5.06-5.37 (m, 4H, Aloc), 5.72 (m, 1H, Aloc), 5.88 (m, 1H, Aloc), 7.10-7.46 (m, 11H, aromatic), 7.64 (d, 2H, ^{3}J=8.3 Hz, aromatic) ppm; MS (FAB) *m/e* 809 (M + Na)$^+$; ; $C_{41}H_{50}N_6O_{10}$ (·½H_2O) calculated C 61.87%, H 6.46%, N 10.56%, measured C 61.84%, H 6.38%, N 10.38%.

Example 12

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PNP **18.**

[0043] A solution of 80.2 mg (102 µmol) of compound **17**, pyridine (25 µl, 3.0 eq.) and 4-nitrophenyl chloroformate (44.3 mg, 220 µmol) in dry tetrahydrofuran/dichloromethane was stirred under an Argon atmosphere at 0°C for two hours and overnight at room temperature. The solution was evaporated in vacuo and the residual product was dissolved in dichloromethane. After washing the organic layer with brine and 0.5 N potassium bisulfate, the organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The resulting crude product was subjected to column chromatography (SiO_2 - $CHCl_3$/MeOH 20/1) to obtain 61.9 mg (84%) of compound **18.** M.P. 69-70°C; ^{1}H-NMR (300

MHz, $CDCl_3/CD_3OD$) : δ 1.23 (d, 3H, ^3J=7.0 Hz, CH_3-Ala), 1.10-2.08 (m, 6H, 3 CH_2-Lys), 3.04 (m, 1H, benzylic), 3.13 (m, 2H, N-CH_2-Lys), 3.27 (bd, 1H, benzylic), 4.06 (m, 1H, Hα), 4.26 (m, 1H, Hα), 4.35-4.70 (m, 5H, Hα and Aloc), 5.04-5.47 (m, 4H, Aloc), 5.14 (s, 2H, benzylic-spacer), 5.24 (s, 2H, benzylic-spacer), 5.72 (m, 1H, Aloc), 5.90 (m, 1H, Aloc), 7.10-7.46 (m, 13H, aromatic), 7.65 (d, 2H, ^3J=8.3 Hz, aromatic), 8.27 (d, 2H, ^3J=9.1 Hz, aromatic-PNP) ppm; MS (FAB) *m/e* 952 $(M + H)^+$, 974 $(M + Na)^+$; $C_{40}H_{46}N_6O_{12}$ (·¼H_2O) calculated C 59.51%, H 5.81%, N 10.41%, measured C 59.52%, H 5.54%, N 10.12%.

Example 13

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-DOX **19.**

[0044] The double spacer containing 4-nitrophenyl carbonate **18** (140 mg, 0.147 mmol) and doxorubicin-HCl (94.1 mg, 1.1 eq.) in N-methyl pyrrolidinone were treated at room temperature with riethyl amine (22.5 µl, 1.1 eq.). The reaction mixture was stirred in the dark for 72 hours, again triethyl amine (1.1 eq.) was added and after an additional 24 hours the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water and brine, and was dried (Na_2SO_4). After evaporation of the solvents the crude product was purified by means of column chromatography (chloroform-methanol; 9:1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate (chloroform-methanol; 9:1), to yield 72 mg (36%) of protected prodrug **19.** M.P. 129 °C; ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.22 (d, 3H, *J* = 7.1 Hz, sugar CH_3), 1.27 (d, 3H, *J* = 6.7 Hz, CH_3-Ala), 1.25-2.00 (m, 8H, CH_2-Lys and 2'), 2.15 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.04 (bd, 1H, *J* = 18.7 Hz, 10), 2.90-3.50 (m, 5H, benzylic Phe and N-CH_2-Lys and 10), 3.37 (bs, 1H, 4'), 3.58 (m, 1H, 3'), 3.85 (m, 1H, Hα), 4.08 (s, 3H, OMe), 4.14 (m, 1H, Hα), 4.29 (dd, 1H, 5'), 4.37-4.68 (m, 5H, Hα and 4 Aloc), 4.76 (s, 2H, 14), 4.96 (s, 2H, benzylic spacer), 5.11 (s, 2H, benzylic spacer), 5.02-5.40 (m, 4H, Aloc), 5.48 (bs, 1H, 1'), 5.61-6.00 (m, 3H, Aloc and 7), 7.08-7.39 (m, 9H, aromatic 5H Phe and 4H spacers), 7.33 (d, 2H, *J* = 8.3 Hz, 2H aromatic spacer), 7.42 (d, 1H, *J* = 8.4 Hz, 3), 7.62 (d, 2H, *J* = 8.0 Hz, 2H aromatic spacer), 7.80 (t, 1H, *J* = 8.1 Hz, 2), 8.03 (d, 1H, *J* = 7.5 Hz, 1) ppm; MS (FAB) *m/e* 1378 $(M + Na)^+$; Anal. ($C_{69}H_{77}N_7O_{22}$·$2H_2O$) calculated C 59.52%, H 5.86%, N 7.04%, measured C 59.34%, H 5.71%, N 6.66%.

Example 14

Synthesis of H-D-Ala-Phe-Lys-PABC-PABC-DOX·5.7HCl **20.**

[0045] To a solution of 48 mg (0.035 mmol) protected prodrug **19** in dry tetrahydrofuran/dichloromethane under an argon atmosphere was added morpholine (31 µl, 10 eq.) together with a catalytic amount of $Pd(PPh_3)_4$. The reaction mixture was stirred for one hour in the dark. The red precipitate was collected by means of centrifugation. Ethyl acetate was added and the mixture was acidified using 1.0 ml of 0.5 M hydrochloric acid/ethyl acetate. The precipitate was collected by means of centrifugation and washed several times with ethyl acetate. Tert-butanol was added and evaporated and the resulting red film was freeze dried in water yielding 37 mg (83%) of prodrug **20.** Mp>300 °C; ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.20 (d, 3H, *J* = 7.0 Hz, sugar CH_3), 1.27 (d, 3H, *J* = 6.5 Hz, CH_3-Ala), 1.38-2.05 (m, 8H, CH_2-Lys and 2'), 2.18 (dd, 1H, 8), 2.36 (bd, 1H, 8), 2.82-3.41 (m, 6H, benzylic Phe and N-CH_2-Lys and 10), 3.37 (s, 1H, 4'), 3.60 (bs, 1H, 3'), 4.02 (m, 1H, Hα), 4.08 (s, 3H, OMe), 4.18 (m, 1H, Hα), 4.53 (dd, 1H, 5'), 4.66 (dd, 1H, Hα), 4.77 (s, 2H, 14), 4.95 (bs, 2H, benzylic spacer), 5.14 (s, 2H, benzylic spacer), 5.27 (bs, 1H, 1'), 5.48 (bs, 1H, 7), 7.09-7.50 (m, 11H, aromatic 5H Phe and 6H spacers and 3), 7.58 (d, 2H, *J* = 8.4 Hz, 2H aromatic spacer), 7.82 (t, 1H, *J* = 8.0 Hz, 2), 8.03 (d, 1H, *J* = 7.6 Hz, 1) ppm; MS (FAB) *m/e* 1188 $(M + H)^+$, *m/e* 1210 $(M + Na)^+$; Anal. (duplo) ($C_{61}H_{69}N_7O_{18}$·5.7HCl) calculated C 52.42%, H 5.39%, N 7.01%, measured C 52.38%, H 5.71%, N 7.14%.

Example 15

Synthesis of 2'-[Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC]-paclitaxel **21.**

[0046] 4-Nitrophenyl carbonate **18** (47.4 mg, 49.8 µmol) and paclitaxel (42.3 mg, 1.0 eq.) in dry tetrahydrofuran/dichloromethane under an Argon atmosphere were treated at room temperature with N,N-dimethyl-4-aminopyridine (DMAP) (6.7 mg, 1.1 eq.). The reaction mixture was stirred in the dark for 48 hours and was then concentrated to dryness. The product was dissolved in dichloromethane and the organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate and brine and dried over anhydrous sodium sulfate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (SiO_2 - EtOAc/Hex/MeOH 5/5/1), to yield 67.5 mg (82%) of the desired protected paclitaxel prodrug **21.** M.P. 137-138°C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.14 (s, 3H, 17), 1.23 (s, 3H, 16), 1.27 (d, 3H, ^3J=7.1 Hz, CH_3-Ala), 1.05-2.10 (m, 6H, CH_2-Lys), 1.67 (s, 3H, 19),

1.89 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.44 (s, 3H, 4-OAc), 2.97 (m, 1H, benzylic), 3.14 (m, 2H, N-CH_2-Lys), 3.21 (m, 1H, benzylic), 3.81 (d, 1H, ^3J=7.0 Hz, 3), 4.03 (m, 1H, Hα), 4.20 (d, 1H, ^2J=8.4 Hz, 20b), 4.31 (d, 1H, ^2J=8.4 Hz, 20a), 4.43 (m, 1H, 7), 4.34-4.74 (m, 6H, Hα and Abc), 4.90-5.37 (m, 11H, 2 Hα, Aloc, 5 and 2 benzylic-spacer), 5.44 (d, 1H, ^3J=2.9 Hz, 2'), 5.63 (m, 1H, Aloc), 5.69 (d, 1H, ^3J=7.1 Hz, 2), 5.87 (m, 1H, Aloc), 5.97 (bd, 1H, ^3J=2.9 Hz, ^3J=9.2 Hz, 3'), 6.26 (m, 1H, 13), 6.29 (m, 1H, 10), 7.05-7.80 (m, 26H, aromatic), 8.14 (d, 2H, ^3J=7.2 Hz, aromatic) ppm; MS (FAB) *m/e* 1668 $(M + H)^+$, 1689 $(M + Na)^+$; $C_{89}H_{99}N_7O_{25}$ (·$2H_2O$) calculated C 62.78%, H 6.10%, N 5.76%, measured C 62.55%, H 5.82%, N 5.57%.

Example 16

Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-PABC]-paclitaxel (·2HCl) **22.**

[0047] To a solution of 51.4 mg (30.8 µmol) protected prodrug **21** in dry tetrahydrofuran under an Argon atmosphere was added glacial acetic acid (8.9 µl, 5 eq.) together with tributyltinhydride (24.6 µl, 3 eq) and a catalytic amount of Pd $(PPh_3)_4$. After 30 minutes the reaction mixture carefully 1 ml 0.5 M HCl/EtOAc was added to the reaction solution. The product was precipitated by addition of diethyl ether and the white precipitate was collected by means of centrifugation and washed several times with ether. Tert-butanol was added and evaporated again to remove an excess of HCl and the resulting product was dissolved in water and freeze dried yielding 46.9 mg (100%) of the desired prodrug **22.** M. P. > 192°C (dec.); ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.15 (s, 3H, 17), 1.21 (s, 3H, 16), 1.10-2.00 (m, 9H, CH_2-Lys and CH_3-Ala), 1.67 (s, 3H, 19), 1.90 (s, 3H, 18), 2.20 (s, 3H, 10-OAc), 2.43 (s, 3H, 4-OAc), 2.85 (m, 4H, benzylic and N-CH_2-Lys), 3.80 (d, 1H, ^3J=6.9 Hz, 3), 4.24 (d, 1H, ^2J=8.4 Hz, 20b), 4.31 (d, 1H, ^2J=8.4 Hz, 20a), 4.39 (dd, 1H, 7), 4.56 (m, 1H, Hα), 5.68 (m, 1H, Hα), 4.98 (d, 1H, 5), 5.08 (m, 4H, 2 benzylic-spacer), 5.43 (d, 1H, ^3J=2.7 Hz, 2'), 5.70 (d, 1H, ^3J=7.0 Hz, 2), 5.97 (m, 1H, 3'), 6.22 (m, 1H, 13), 6.32 (m, 1H, 10), 7.05-7.68 (m, 24H, aromatic), 7.71 (d, 1H, ^3J=7.2 Hz, aromatic), 8.14 (d, 2H, ^3J=7.3 Hz, aromatic) ppm; MS (FAB) *m/e* 1499 $(M + H)^+$, 1521 $(M + Na)^+$; $C_{81}H_{91}N_7O_{21}$ (·3.7HCl) calculated C 59.60%, H 5.85%, N 6.01%, measured C 59.60%, H 5.88%, N 5.98%.

Example 17

Synthesis of Fmoc-Trp-Lys(Boc)-OBu **24.**

[0048] To a solution of 3.00 g (5.73 mmol) Fmoc-Trp-ONSu **23** in dry dichloromethane under an argon atmosphere were added at 0 °C 0.791 ml (1.00 equiv) triethylamine and 2.12 g (1.10 equiv) H-Lys (Boc)-OBu·HCl. The mixture was stirred at rt for 5 hours, then dichloromethane was added and the organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water, dried over sodium sulfate and evaporated. The white solid **24** (3.52 g, 86%) was used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.10-1.92 (m, 24H, 3 CH_2-Lys and 18 *t*-Bu), 2.80-3.20 (m, 3H, N-CH_2-Lys and CH_2-Trp), 3.52 (d, 1H, CH_2-Trp), 4.19 (t, 1H, Fmoc), 4.29-4.82 (m, 5H, 2 Fmoc, 2 Hα and NH), 6.54 (d, H, Aryl), 7.06-7.76 (m, 12H, aromatic) ppm; MS (FAB) *m/e* 1444 (2M + Na); Anal. ($C_{41}H_{50}N_4O_7$·$4H_2O$) C, H, N calculated C 62.90%, H 6.30%, N 7.15%, measured C 63.22%, H 6.49%, N 7.13%.

Example 18

Synthesis of Boc-D-Ala-Trp-Lys(Boc)-OBu **26.**

[0049] 3.52 g (4.95 mmol) of Fmoc-Trp-Lys(Boc)-OBu **24** was dissolved in 100 ml of dioxane/methanol/2N sodium hydroxide (70/25/5) and stirred at rt for 1 hour. The mixture was neutralized with acetic acid (0.570 ml) and organic solvents were evaporated. Water and dioxane were added and the solution was freeze dried. Diisopropylether was added and after filtration the filtrate was evaporated. The product was dissolved in dry dichloromethane and added at 0 °C to a solution of 1.41 g (4.93 mmol) Boc-D-Ala-ONSu **6** and 0.756 ml (1.10 equiv) of triethylamine in dry dichloromethane. The mixture was stirred for 16 hours after which dichloromethane was added. The organic layer was washed with 10% citric acid, saturated sodium bicarbonate and water, and dried over sodium sulfate and evaporated. The product was purified by means of column chromatography ((SiO_2 - first ethyl acetate/ heptane 1/1 and then $CHCl_3$/MeOH; 9/1) to afford 2.26 g (3.42 mmol, 69%) of the tripeptide **26** as white foam. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.99-1.90 (m, 36 H, 3 CH_2-Lys, CH_3-Ala and 3 *t*-Bu), 2.80-3.50 (m, 4H, N-CH_2-Lys and 2 CH_2-Trp), 3.99 (m, 1H, Hα), 4.33 (m, 1H, Hα), 4.77 (br d, 1H, Hα), 6.90-7.65 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 660 $(M + H)^+$, 682 $(M + Na)^+$; Anal. ($C_{34}H_{53}N_5O_8$·H_2O) C, H, N calculated C 60.25%, H 8.17%, N 10.33%, measured C 60.47%, H 8.08%, N 9.73%.

Example 19

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-OH **28.**

[0050] 2.56 g (4.13 mmol) Boc-D-Ala-Trp-Lys (Boc)-OBu **(26)** was stirred in a solution of hydrochloric acid in ethyl acetate (3M). After 5 hour the solvent was evaporated, tert-butanol was added and evaporated twice to remove remaining hydrochloric acid. The product was freeze dried in dioxane/water to yield a brown coloured powder. To a solution of 706 mg (1.61 mmol) D-Ala-Phe-Lys-OH **27** in water/acetonitrile was added triethylamine until a pH of 9 - 9.5 was reached. Then a solution of 1.58 g (2.20 equiv) Aloc-ONSu in acetonitrile was added and the mixture was kept basic by adding triethylamine. After the pH of the mixture did not alter anymore, a 0.5 M solution of hydrochloric acid in ethyl acetate was added until a pH of 3 was reached. The mixture was thoroughly extracted with dichloromethane. The organic layer was washed with water, dried (Na_2SO_4) and evaporated. The cream coloured product **28** was used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00-1.80 (m, 9H, 3 CH_2-Lys and CH_3-Ala), 2.80-3.35 (m, 4H, N-CH_2-Lys and CH_2Trp), 4.13 (m, 1H, Hα), 4.14 (m, 1H, Hα), 4.30-4.95 (m, 6H, 4 Aloc and 2 H α), 5.01-5.40 (m, 5H, 4 Aloc and Hα), 5.70-6.30 (m, 3H, 2 Aloc and NH), 6.90-7.70 (m, 5H, aromatic) ppm; MS (FAB) *m/e* 572 $(M + H)^+$, 594 $(M + Na)^+$; Anal. ($C_{28}H_{37}N_5O_8 \cdot 1½H_2O$) calculated C 56.18%, H 6.44%, N 11.70%, measured C 56.07%, H 6.22%, N 11.21%.

Example 20

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABA **29.**

[0051] A solution of 239 mg (0.419 mmol) Aloc-D-Ala-Trp-Lys(Aloc)-OH **28** was dissolved in dry tetrahydrofuran under an argon atmosphere and cooled to -40 °C. N-methylmorpholine (48.3 µl, 1.05 equiv) and isobutylchloroformate (57.0 µl, 1.05 equiv) were added. The reaction mixture was stirred for 2 hours at a temperature below -30 °C. A solution of 4-aminobenzyl alcohol (51.5 mg, 1.00 equiv) and N-methylmorpholine (50.6 µl, 1.1 equiv) in dry THF was added dropwise to the reaction mixture. After 2 hours tetrahydrofuran was evaporated and dichloromethane was added. The organic layer was washed with saturated sodium bicarbonate, a 0.5 N potassium bisulphate solution and brine, dried (Na_2SO_4) and evaporated to afford 265 mg (94%) of the desired product **29** as a cream coloured powder. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.00-1.62 (m, 9H, CH_3-Ala and 3 CH_2-Lys), 2.90-3.70 (m, 4H, N-CH_2-Lys and CH_2-Trp), 4.48-4.92 (m, 7H, 2 Hα and 4 Aloc), 4.72 (s, 2H, CH_2-OH), 5.00-5.50 (m, 5H, 4 Aloc and Hα), 5.35-6.05 (m, 2H, Aloc), 6.80-7.83 (m, 9H, aromatic) ppm; MS (FAB) *m/e* 677 $(M + H)^+$, 699 $(M + Na)^+$.

Example 21

Synthesis of Aloc-D-Ala-Trp-Lys(Abc) -PABC-PNP.

[0052] To a solution of 384 mg (0.602 mmol) of Aloc-D-Ala-Trp-Lys(Aloc)-PABA **29** in dry tetra hydro furan/dichloromethane under an argon atmosphere, 4-nitrophenylchloroformate (182 mg, 1.50 equiv) and dry pyridine (73 µl, 1.50 equiv) were added. The mixture was stirred at rt for 48 hours, and then ethyl acetate was added. The organic layer was washed with 10% citric acid, brine and water, dried (Na_2SO_4) and evaporated yielding a yellow solid. The product was purified by means of column chromatography (SiO_2 -$CHCl_3$/MeOH; 30/1) to afford 324 mg (67%) of carbonate Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PNP as a cream coloured powder. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$): δ 1.00-2.10 (m, 9H, CH_3-Ala and 3 CH_2-Lys), 2.90-3.70 (m, 4H, N-CH_2-Lys and CH_2-Trp), 3.64 (m, 1H, Hα), 3.81 (m, 1H, Hα), 4.38-4.81 (m, 5H, Hα and 4 Aloc), 5.10-5.35 (m, 4H, Aloc), 5.21 (s, 2H, CH_2-OH), 5.40-6.00 (m, 2H, Aloc), 7.00-7.85 (m, 11H, aromatic), 8.25 (d, 2H, J = 8.1, aromatic) ; MS (FAB) *m/e* 842 $(M + H)^+$, 864 $(M + Na)^+$.

Example 22

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABA **30.**

[0053] To a solution of 219 mg (260 µmol) of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PNP and 35.2 mg (1.1 equiv) 4-aminobenzyl alcohol in dry N,N-dimethylformamide under an Argon atmosphere was added diisopropylethylamine (45.3 µl, 1.00 equiv) and a catalytic amount of N-hydroxybenzotriazole (10.5 mg, 0.30 equiv). The reaction solution was stirred for 48 hours after which it was diluted with 10% propanol-2/EtOAc. The organic layer was washed with saturated sodium bicarbonate, 0.5 N potassium bisulfate and brine, dried over anhydrous sodium sulfate and evaporated to dryness. The pale yellow residual film was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH 15/1) to yield 192 mg (89%) of the desired product **30.** ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.90-2.10 (m, 9H, CH_3-Ala and 3 CH_2-

Lys), 2.90-3.70 (m, 4H, N-CH_2-Lys and CH_2-Trp), 4.08 (m, H, Hα), 4.40-4.86 (m, 6H, 2 benzylic-spacer and 4 Aloc), 4.90-5.40 (m, 7H, 2 benzylic-spacer Hα and Aloc), 5.50 (m, 1H, Aloc), 5.92 (m, 1H, Aloc), 6.72-7.82 (m, 13H, aromatic) ppm; MS (FAB) *m/e* 848 $(M + Na)^+$; ($C_{43}H_{51}N_7O_{10} \cdot 2^3/_4H_2O$) calculated C 58.99%, H 6.50%, N 11.20%, measured C 59.15%, H 6.25%, N 11.15%.

Example 23

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABC-PNP **31.**

[0054] A solution of 70 mg (85 µmol) of compound **30,** pyridine (17 µl, 2.5 equiv) and 4-nitrophenylchloroformate (34 mg, 2.0 equiv) was stirred under an Argon atmosphere at 0°C for two hours and for 24 hours at room temperature. The solution was evaporated in vacuo and the residual product was dissolved in chloroform. After washing the organic layer with brine and 0.5 N potassium bisulfate, the organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The resulting crude product was subjected to column chromatography (SiO_2 - $CHCl_3$/MeOH 20/1) to obtain 54 mg (64%) of **31** as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 0.90-2.10 (m, 9H, CH_3-Ala and 3 CH_2-Lys), 2.90-3.10 (m, 4H, N-CH_2-Lys and CH_2-Trp), 3.27 (bd, 1H, benzylic), 4.35-4.78 (m, 6H, 2Hα and Aloc), 4.90-5.52 (m, 4H, Aloc), 5.13 (s, 2H, benzylic-spacer), 5.60 (m, 1H, Aloc), 5.94 (m, 1H, Aloc), 7.10-7.46 (m, 15H, aromatic), 8.36 (d, 2H, aromatic-PNP) ppm; MS (FAB) *m/e* 991 $(M + H)^+$, 1013 $(M + Na)^+$; $C_{50}H_{54}N_8O_{14} \cdot ¾H_2O$) calculated C 59.78%, H 5.57%, N 11.15%, measured C 60.12%, H 5.89%, N 10.76%.

Example 24

Synthesis of Aloc-D-Ala-Trp-Lys(Aloc)-PABC-PABC-DOX **32.**

[0055] The double spacer-containing 4-nitrophenylcarbonate **31** (41 mg, 0.041 mmol) and doxorubicin-HCl (26 mg, 1.1 equiv) in N-methylpyrrolidinone were treated at room temperature with triethylamine (6.3 µl, 1.1 equiv). The reaction mixture was stirred in the dark for 48 hours, again triethylamine (1.1 equiv) was added and after an additional 24 hours the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water and brine, and was dried (Na_2SO_4). After evaporation of the solvents the crude product was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH; 9/1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate (chloroform-methanol; 9/1), to yield 45 mg (78%) of the protected prodrug **32.** ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 0.92-1.52 (m, 13H, sugar CH_3, CH_3-Ala, 3 CH_2-Lys and 2'), 2.15 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.18 (bd, 1H, 10), 2.90-3.10 (m, 5H, N-CH_2-Lys and CH_2-Trp and 10), 3.59 (bs, 1H, 4'), 3.82 (m, 1H, 3'), 3.85 (m, 1H, Hα), 4.11 (s, 3H, OMe), 4.21 (m, 1H, Hα), 4.45 (dd, 1H, 5'), 4.30-4.62 (m, 5H, Hα and 4 Aloc), 4.76 (s, 2H, 14), 4.96 (s, 2H, benzylic spacer), 5.11 (s, 2H, benzylic spacer), 5.513-5.4 (m, 2H, Aloc), 5.48 (bs, 1H, 1'), 5.58 (m, 2H, Aloc and 7), 5.91(m, 2H, Aloc), 6.70-7.39 (m, 11H, aromatic 5 Trp and 6 spacers), 7.41 (d, 1H, J = 8.4 Hz, 3), 7.63 (d, 2H, aromatic spacer), 7.78 (t, 1H, 2), 8.03 (d, 1H, J = 7.6 Hz, 1) ppm; MS (FAB) *m/e* 1417 $(M + Na)^+$.

Example 25

Synthesis of D-Ala-Trp-Lys-PABC-PABC-DOX (·7½ HCl) **33.**

[0056] To a solution of 36 mg (0.026 mmol) protected prodrug **32** in dry THF/dichloromethane under an argon atmosphere was added morpholine (22 µl, 10 equiv) together with a catalytic amount of $Pd(PPh_3)_4$. The reaction mixture was stirred for 1 hour in the dark. The red precipitate was collected by means of centrifugation. Ethyl acetate was added and the mixture was acidified using 0.5 ml of 1 M hydrochloric acid/ethyl acetate. The precipitate was collected by means of centrifugation and washed several times with ethyl acetate. Tert-butanol was added and evaporated and the resulting red film was freeze dried in water yielding 28 mg (72%) of the doxorubicin prodrug **33.** ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.10-1.96 (m, 13H, CH_3-Ala, CH_3-sugar, 3 CH_2-Lys and 2'), 2.09 (m, 1H, 8), 2.35 (bd, 1H, J = 15.1 Hz, 8), 2.79-3.39 (m, 3H, N-CH_2-Lys, CH_2-Trp and 10), 3.60 (s, 1H, 4'), 4.00 (bs, 1H, 3'), 4.09 (s, 3H, OMe), 4.54 (m, 1H, 5'), 4.77(s, 2H, 14), 4.97 (2 * d, 2H, Bn spacer), 5.13(s, CH_2, Bn spacer), 5.28 (bs, 1H, 1'), 5.48 (bs, 1H, 7), 6.99-7.72 (m, 12H, 5 Trp and 6 spacer), 7.62(d, 1H, 7.6 Hz, 3), 7.55 (d, 2H, J = 8.2 Hz, aromatic spacer), 7.83 (t, 1H, 2), 8.05 (d, 1H, J = 7.7 Hz, 1) ppm; MS (FAB) *m/e* 1228 $(M + H)^+$; Anal. ($C_{63}H_{70}N_8O_{18} \cdot 7\ ½HCl$) calculated C 50.42%, H 5.21%, N 7.47%, measured C 50.56%, H 5.48%, N 7.35%.

Example 26

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABA **34.**

[0057] 100 mg (0.105 mmol) of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PNP **18** was dissolved in dry N,N-dimethylformamide under an argon atmosphere and cooled to -8 °C. 4-Aminobenzylalcohol (14.2 mg, 1.1 equiv), dipea (18.3 µl, 1.0 equiv) and 1-hydroxybenzotriazole (HOBt) (4 mg, 0.3 equiv) were added. The reaction mixture was stirred for 48 hours at room temperature, and diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water, saturated sodium bicarbonate, 0.5 N potassium bisulfate, and brine, dried over sodium sulfate (Na_2SO_4), and evaporated to yield the desired product **34** as a cream colored powder 86 mg (88%). ^{1}H NMR (300 MHz $CDCl_3$) δ 0.95-2.05 (m, 9H, 3CH_2-Lys and CH_3-Ala), 2.88-3.11 (m , 4H, 2H Bn-Phe and N-CH_2-Lys), 3.95-4.62 (m, 7H, 3Hα and 4H Aloc), 4.75 (s, 2H, CH_2-OH), 5.12-5.21 (m, 6H, 4 Aloc and CH_2-Bn), 5.09 (s, 2H, CH_2-Bn), 5.65-6.00 (m, 2H, Aloc), 6.79-7.41 (m, 15H, aromatic) 7.62 (d, 2H, aromatic) ppm; MS (FAB) m/e 959 $(M + Na)^+$.

Example 27

Synthesis of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABC-PNP **35.**

[0058] To a solution of 59 mg (0.063 mmol) of Aloc-D-Ala-Phe-Lys(Aloc)-PABC-PABC-PABA **34** in dry tetrahydrofuran and dichloromethane under an argon atmosphere, were added at -40 °C respectively pyridine (13 µl, 2.5 equiv) and 4-nitrophenyl chloroformate (25 mg, 2.0 equiv). After stirring for 4.5 hours at -40 °C and overnight at 6 °C, pyridine (10 µl, 2.0 equiv) and 4-nitrophenylchloroformate (25 mg, 2.0 equiv) were added again. This was repeated after 48 hours stirring at 6 °C. After another 48 hours the solution was evaporated in vacuo and the residual product was dissolved in chloroform. The organic layer was washed with 10% citric acid, brine and water, dried over sodium sulfate (Na_2SO_4) and evaporated yielding a yellow solid. The crude product was purified by means of column chromatography (SiO2-$CHCl_3$ /MeOH; 15/1) to give the desired product **35** quantitatively. ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$): δ 1.12-1.89 (m, 9H, CH_3-Ala and 3 CH_2-Lys), 3.04 (m, 1H, benzylic), 3.14 (m, 2H, N-CH_2-Lys), 3.27 (bd, 1H, benzylic), 4.09 (m, 1H, Hα), 4.28 (m, 1H, Hα), 4.34-4.68 (m, 5H, Hα and Aloc), 5.02-5.40 (m, 4H, Aloc), 5.14 (s, 2H, benzylic-spacer), 5.21 (s, 2H, benzylic-spacer), 5.31 (s, 2H, benzylic- spacer), 5.72 (m, 1H, Aloc), 5.90 (m, 1H, Aloc), 7.10-7.52 (m, 17H, aromatic), 7.63 (d, 2H, J=8.3 Hz, aromatic), 8.27 (d, 2H, J=9.1 Hz, aromatic-PNP) ppm; MS (FAB) *m/e* 1102 $(M + H)^+$, 1124 $(M + Na)^+$.

Example 28

Synthesis of Aloc-D-Ala-Phe-Lys(Abc)-PABC-PABC-PABC-DOX **36.**

[0059] The 4-nitrophenyl carbonate **35** (69 mg, 0.063 mmol) and doxorubicin-HCl (40 mg, 1.1 equiv) in N-methylpyrrolidinone were treated at room temperature with triethylamine (9.7 µl, 1.1 equiv). The reaction mixture was stirred in the dark for 24 hours and the reaction mixture was diluted with 10% 2-propanol/ethyl acetate. The organic layer was washed with water and brine, and was dried over sodium sulfate (Na_2SO_4). After evaporation of the solvents the crude product was purified by means of column chromatography (SiO_2- $CHCl_3$/MeOH 9/1) followed by circular chromatography using a chromatotron supplied with a 2 mm silica plate ($CHCl_3$/MeOH; 9/1), to yield 65 mg (71%) of the protected prodrug **36.** ^{1}H-NMR (300 MHz, $CDCl_3/CD_3OD$) : δ 1.10-1.80 (m, 14H, sugar CH_3, CH_3-Ala, 3 CH_2-Lys and 2'), 2.14 (dd, 1H, 8), 2.36 (bd, 1H, 8), 3.18 (bd, 1H, 10), 2.82-3.41 (m, 6H, benzylic Phe and N-CH_2-Lys and 10), 3.37 (s, 1H, 4'), 3.60 (bs, 1H, 3'), 4.02 (m, 1H, Hα), α), 4.07 (s, 3H, OMe) 4.29 (dd, 1H, 5'), 4.37-4.68 (m, 5H, Hα and 4 Aloc), 4.76 (s, 2H, 14), 4.95 (bs, 2H, benzylic spacer), 5.10 (s, 2H, benzylic spacer), 5.14 (s, 2H, benzylic spacer), 5.02-5.35 (m, 4H, Aloc), 5.27 (bs, 1H, 1'), 5.47 (bs, 1H, 7), 5.70 (m, 1H, Aloc), 5.89 (m, 1H, Aloc), 7.09-7.50 (m, 16H, 5 Phe and 10 spacers and 3), 7.64 (d, 2H, J = 8.4 Hz, 2H aromatic spacer), 7.79 (t, 1H, J = 8.1 Hz, 2), 8.06 (d, 1H, J = 7.5 Hz, 1) ppm; MS (FAB) *m/e* 1506 $(M + H)^+$, 1528 $(M + Na)^+$.

Example 29

Synthesis of D-Ala-Phe-Lys-PABC-PABC-PABC-DOX (·2HCl) **37.**

[0060] To a solution of 40 mg protected prodrug **36** (0.027 mmol) in dry tetrahydrofuran/dichloromethane under an argon atmosphere were added morpholine (24 µl, 10 equiv) and a catalytic amount of $Pd(PPh_3)_4$. The reaction mixture was stirred for 1 hour in the dark. The red precipitate was collected by means of centrifugation and washed several times with ethyl acetate. Water and dioxane were added and the mixture was acidified using 4.4 ml of 0.125 mM

hydrochloric acid. After freeze drying 26 mg (70%) of doxorubicin prodrug **37** was obtained. ^{1}H-NMR (300 MHz, $CDCl_3$/CD_3OD) : δ 1.19 (d, 3H, *J* = 6.9 Hz, sugar CH_3), 1.27 (d, 3H, *J* = 6.6 Hz, CH_3-Ala), 1.25-2.00 (m, 8H, 3 CH_2-Lys and 2'), 2.18 (dd, 1H, 8), 2.33 (br d, 1H, *J* = 16.1 Hz, 8), 2.89-3.38 (m, 6H, N-CH_2-Lys and 10 and Bn Phe), 3.60 (s, 1H, 4'), 3.72 (m, 1H, 3'), 4.08 (s, 3H, OMe), 4.18 (m, 1H, Hα), 4.53 (dd, 1H, 5') 4.66 (m, 1H, Hα), 4.77 (s, 2H, 14), 4.96 (s, 2H, Bn spacer), 5.11 (s, 2H, bn spacer), 5.17 (s, 2H, Bn spacer), 5.27 (br s, 1H, 1'), 5.48 (br s, 1H, 7), 7.05-7.35 (m, 16H, aromatic spacer and 3), 7.52 (d, 2H, *J* = 8.5 Hz, aromatic spacer), 7.84 (t, 1H, 2), 8.01 (d, 1H, *J* = 7.7 Hz, 1) ppm.

Example 30

Synthesis of 2'-[4-nitrophenyl carbonate]-paclitaxel **38.**

[0061] To a solution of 194 mg (0.227 mmol) paclitaxel in dry dichloromethane under an Argon atmosphere was added pyridine (4 drops). At -50 °C, 275 mg (6.0 eq.) 4-nitrophenyl chloroformate dissolved in dry dichloromethane was added. The reaction mixture was stirred at -50 °C and after 4 hours 4-nitrophenyl chloroformate (4.2 eq.) was added. After 1 hour the mixture was diluted with dichloromethane and washed with 0.5 N potassium bisulfate and brine and dried over anhydrous sodium sulphate. After evaporation of the solvents the residual yellow film was purified by means of column chromatography (SiO_2 - EtOAc/Hex 1/1), to yield 133 mg of activated paclitaxel **38** (78%, 73% conversion). M.P. 161°C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.14 (s, 3H, 17), 1.25 (s, 3H, 16), 1.68 (s, 3H, 19), 1.92 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.49 (s, 3H, 4-OAc), 2.55 (m, 1H, 6a), 3.82 (d, 1H, 3), 4.21 (d, 1H, 20b), 4.32 (d, 1H, 20a), 4.42 (m, 1H, 7), 4.96 (bd, 1H, 5), 5.53 (d, 1H, 2'), 5.69 (d, 1H, 2), 6.09 (q, 1H, 3'), 6.29 (s, 1H, 10), 6.34 (m, 1H, 13), 6.90 (d, 1H, N-H), 7.20-7.65 (m, 13H, aromatic), 7.75 (d, 2H, aromatic), 8.15 (d, 2H, aromatic), 8.25 (d, 2H, nitrophenyl) ppm; MS (FAB) *m/e* 1020 $(M + H)^+$, 1042 $(M + Na)^+$; $C_{54}H_{54}N_2O_{19}$ (·1½H_2O) calculated C 62.00%, H 5.40%, N 2.68%, measured C 61.89%, H 5.52%, N 2.64%.

Example 31

Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-N(Me)-$(CH_2)_2$-N(Me)CO] - paclitaxel (·2HCl) **43.**

Step a: Synthesis of N(Me)-$(CH_2)_2$-N(Me)-Z **39** (Z = benzyloxy carbonyl).

[0062] To a solution of 1.21 g (13.7 mmol) N,N'-dimethyl ethylenediamine in dry dichloromethane under an Argon atmosphere at room temperature was added dropwise a solution of Z-ONSu (338 mg, 1.36 mmol) in dry dichloromethane. After stirring for 120 minutes the solution was concentrated in vacuo. The residual product was dissolved in ethyl acetate and the organic layer was washed with brine. The organic solvent was dried over anhydrous sodium sulfate and evaporated to dryness. The oily product was purified by means of column chromatography (SiO_2 - $CHCl_3$/MeOH 1/1) to obtain 249 mg (83%) of the product **39** as an oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.42 (bd, 3H, ^{3}J=13.9 Hz, CH_3-NH-CH_2), 2.73 (m, 2H, CH_3-NH-CH_2), 2.95 (s, 3H, CH_3-N), 3.41 (bs, 2H, CH_2-N), 5.13 (s, 2H, CH_2-Z), 7.25-7.40 (m, 5H, aromatic) ppm.

Step b: Synthesis of 2'-[Z-N(Me)-$(CH_2)_2$-N(Me)CO]- paclitaxel **40.**

[0063] To a solution of 114 mg (112 μmol) 2'-activated paclitaxel **38** and 25 mg Z-protected N,N'-dimethyl ethylenediamine **39** in dry dichloromethane under an Argon atmosphere at -50°C was added triethyl amine (20.0 μl, 144 μmol). The solution was stirred 7 hours at -40°C, subsequently allowed to heat up to room temperature and then stirring was continued overnight at room temperature. The solution was diluted with dichloromethane and washed with saturated sodium bicarbonate, brine and 0.5 N potassium bisulfate. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to obtain a yellow film. The product was purified by column chromatography (SiO_2 - EtOAc/Hex 2/1) to obtain 113 mg (92%) of the desired product **40.** M.P. 130-131°C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.12 (s, 3H, 17), 1.21 (s, 3H, 16), 1.70 (s, 3H, 19), 2.00 (s, 3H, 18), 2.26 (s, 3H, 10-OAc), 2.60 (s, 3H, 4-OAc), 2.90 (s, 3H, CH_3-spacer), 2.94 (s, 3H, CH_3-spacer), 2.97 (m, 1H, CH_2-spacer), 3.06 (m, 1H, CH_2-spacer), 3.54 (m, 1H, CH_2-spacer), 3.78 (m, 1H, CH_2-spacer), 3.84 (d, 1H, ^{3}J=7.2 Hz, 3), 4.23 (d, 1H, ^{2}J=8.4 Hz, 20b), 4.32 (d, 1H, ^{2}J=8.4 Hz, 20a), 4.47 (m, 1H, 7), 4.69 (d, 1H, ^{2}J=12.4 Hz, benzylic), 4.85 (d, 1H, ^{2}J=12.4 Hz, benzylic), 5.01 (m, 1H, 5), 5.47 (d, 1H, ^{3}J=2.9 Hz, 2'), 5.68 (d, 1H, ^{3}J=7.0 Hz, 2), 6.19 (dd, 1H, ^{3}J=9.8 Hz, ^{3}J=2.9 Hz, 3'), 6.28 (s, 1H, 10), 6.33 (m, 1H, 13), 6.94-7.70 (m, 16H, aromatic), 7.83 (d, 2H, ^{3}J=7.3 Hz, aromatic), 8.16 (d, 2H, ^{3}J=7.1 Hz, aromatic), 8.57 (d, 1H, ^{3}J=9.8 Hz, NH) ppm; MS (FAB) *m/e* 1102 $(M + H)^+$, 1124 $(M + Na)^+$; $C_{60}H_{67}N_3O_{17}$ (·H_2O) calculated C 64.33%, H 6.21%, N 3.73%, measured C 64.65%, H 6.11%, N 3.76%.

Step c: Synthesis of 2'- [N(Me) - (CH_2)2-N(Me)CO]- paclitaxel (·7AcOH) **41**.

[0064] To a solution of 61.8 mg (56.1 µmol) of **40** in 5% acetic acid/methanol was added a catalytic amount of 10% Pd-C. The mixture was stirred for 1 hour under a H_2 atmosphere. The Pd-C was removed by means of centrifugation, methanol was evaporated in vacuo, and ethyl acetate was added. The organic layer was extracted with water. The water layer was freeze dried yielding 78.0 mg (100%) of the desired product **41**.

Step d: Synthesis of 2'-[Aloc-D Ala-Phe-Lys(Aloc)-PABC-N(Me)$(CH_2)_2$-N(Me)CO]- paclitaxel **42**.

[0065] To a solution of 152 mg (95.8 µmol) of paclitaxel-spacer compound **41** and 80.7 mg (101 µmol) of carbonate **16** in dry tetrahydrofuran under an Argon atmosphere was added triethyl amine (200 µl, 1.44 mmol). After 24 hours the solution was concentrated to dryness and the residual product was dissolved in dichloromethane and washed with saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product was subjected to column chromatography (SiO_2 - EtOAc/Hex/MeOH 5/5/1) to obtain 113 mg (72%) of the desired protected prodrug **42**. M.P. 127-128°C; ^{1}H-NMR (300 MHz, $CDCl_3$) : δ 1.13 (s, 3H, 17), 1.22 (s, 3H, 16), 1.27 (d, 3H, ^{3}J=5.6 Hz, CH_3-Ala), 1.04-2.00 (m, 6H, CH_2-Lys), 1.69 (s, 3H, 19), 2.00 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.59 (s, 3H, 4-OAc), 2.90 (s, 3H, CH_3-spacer), 2.91 (s, 3H, CH_3-spacer), 2.76-3.46 (m, 6H, CH_2-spacer, benzylic and N-CH_2-Lys), 3.54 (m, 1H, CH_2-spacer), 3.74 (m, 1H, CH_2-spacer), 3.84 (d, 1H, ^{3}J=7.0 Hz, 3), 4.00-5.00 (m, 3H, 3 Hα), 4.23 (d, 1H, ^{2}J=8.4 Hz, 20b), 4.32 (d, 1H, ^{2}J=8.4 Hz, 20a), 4.48 (m, 1H, 7), 4.62 (d, 1H, ^{2}J=12.3 Hz, benzylic), 4.83 (d, 1H, ^{2}J=12.4 Hz, benzylic), 4.30-4.73 (m, 4H, Aloc), 4.93-5.39 (m, 5H, Aloc and 5), 5.48 (d, 1H, ^{3}J=2.9 Hz, 2'), 5.69 (d, 1H, ^{3}J=7.0 Hz, 2), 5.54-5.78 (m, 1H, Aloc), 5.88 (m, 1H, Aloc), 6.18 (bd, 1H, 3'), 6.30 (s, 1H, 10), 6.33 (m, 1H, 13), 7.05-7.78 (m, 20H, aromatic), 7.82 (d, 2H, ^{3}J=7.4 Hz, aromatic), 8.16 (d, 2H, ^{3}J=7.2 Hz, aromatic) ppm; MS (FAB) *m/e* 1653 $(M + Na)^+$; $C_{86}H_{102}N_8O_{24}$ calculated C 62.61%, H 6.35%, N 6.79%, measured C 62.40%, H 6.31%, N 6.36%.

Step e: Synthesis of 2'-[H-D-Ala-Phe-Lys-PABC-N(Me)-$(CH_2)_2$-N(Me)CO] paclitaxel (·2HCl) **43**.

[0066] To a solution of 83.0 mg (50.9 µmol) protected prodrug **42** in dry tetrahydrofuran under an Argon atmosphere was added glacial acetic acid (12 µl, 4.0 eq.) together with tributyltinhydride (41 µl, 3.0 eq) and a catalytic amount of $Pd(PPh_3)_4$. After 30 minutes the product was precipitated by addition of diethyl ether. The white precipitate was collected by means of centrifugation and washed several times with diethyl ether. Tert-butanol was added and evaporated again to remove an excess of HCl and the resulting product was dissolved in water/dioxane and freeze dried yielding 56 mg (70%) of prodrug **43**. M.P. 142°C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.13 (s, 3H, 17), 1.21 (s, 3H, 16), 1.26 (d, 3H, ^{3}J=6.6 Hz, CH_3-Ala), 1.05-2.00 (m, 6H, CH_2-Lys), 1.69 (s, 3H, 19), 2.00 (s, 3H, 18), 2.22 (s, 3H, 10-OAc), 2.58 (s, 3H, 4-OAc), 2.89 (s, 3H, CH_3-spacer), 2.91 (s, 3H, CH_3-spacer), 2.67-3.64 (m, 3H, CH_2-spacer), 2.95 (m, 1H, benzylic), 3.07 (m, 2H, N-CH_2-Lys), 3.15 (m, 1H, benzylic), 3.78 (m, 1H, CH_2-spacer), 3.83 (d, 1H, ^{3}J=7.1 Hz, 3), 4.10-5.05 (m, 2H, 2 Hα), 4.22 (d, 1H, ^{2}J=8.4 Hz, 20b), 4.32 (d, 1H, ^{2}J=8.4 Hz, 20a), 4.46 (m, 1H, 7), 4.60 (m, 1H, Hα), 4.65 (d, 1H, ^{2}J=12.3 Hz, benzylic-spacer), 4.80 (d, 1H, ^{2}J=12.4 Hz, benzylic-spacer), 4.99 (bd, 5H, ^{3}J=7.4 Hz, 5), 5.47 (d, 1H, ^{3}J=2.9 Hz, 2'), 5.68 (d, 1H, ^{3}J=6.9 Hz, 2), 6.17 (bd, 1H, ^{3}J=2.9 Hz, ^{3}J=9.6 Hz, 3'), 6.30 (s, 1H, 10), 6.31 (m, 1H, 13), 7.05-7.70 (m, 20H, aromatic), 7.82 (d, 2H, ^{3}J=7.5 Hz, aromatic), 8.16 (d, 2H, ^{3}J=7.2 Hz, aromatic), 8.54 (d, 1H, ^{3}J=9.6 Hz, NH-paclitaxel) ppm; MS (FAB) *m/e* 1463 $(M + H)^+$, 1485 $(M + Na)^+$; $C_{85}H_{97}N_7O_{22}$ (·3AcOH) calculated C 61.04%, H 6.50%, N 6.71%, measured C 60.91%, H 6.45%, N 7.10%.

Example 32

Stability of both double spacer containing paclitaxel prodrugs **22** and **43**.

[0067] The prodrugs were incubated at concentrations of 150 µM in 0.1 M Tris/HCl buffer (pH 7.3) for 3 days and showed no formation of degradation products (TLC, RP_{18}; CH_3CN/H_2O/AcOH 19/19/2).

Stability of the double spacer containing doxorubicin prodrug **20**.

[0068] The prodrug was incubated at a concentration of 100 - 270 µM in 0.1 M Tris/HCl buffer (pH 7.3) for 90 hours and showed no formation of degradation products (TLC, RP_{18}; CH_3CN/H_2O/AcOH 19/19/2).

Example 33

Enzymatic hydrolysis of the double spacer containing prodrugs by plasmin.

[0069] Hydrolysis of the doxorubicin prodrugs was investigated by incubation at a prodrug concentration of 100 μM in 0.1 M Tris/hydrochloric acid buffer (pH 7.3) in the presence of 50 or 20 μg/mL human plasmin (Fluka). Analysis was carried out with the following HPLC system using a Chrompack Microsphere-C18 column (3 μm, 2 x 100 x 4.6 mm). Elution of the analytical column was performed using 7:3 methanol/50 mM Et_3N-formate buffer (pH 3.0). Detection was performed using an UV-detector (λ = 500 nm).

	[prodrug] (μM)	[plasmin] (μg/mL)	$T_{½}$ activation (min)
Prodrug **44**	100	50	19
Prodrug **44**	200	20	> 75
Prodrug **20**	200	20	12

[0070] Hydrolysis of the paclitaxel prodrugs was investigated by incubation at a prodrug concentration of 200 μM in 0.1 M Tris/hydrochloric acid buffer (pH 7.3) in the presence of 100 μg/mL human plasmin (Fluka). All double spacer containing paclitaxel prodrugs were converted to yield the corresponding parent drug. Capillary electrophoresis was carried out with a CE Ext. Light Path Capillary (80.5 cm, 50 μm), with 1:1 methanol/0.05 M sodium phosphate buffer (pH 7.0) as eluent. Detection was performed at 200 and 254 nm.

	[prodrug] (μM)	[plasmin] (μg/mL)	$T_{½}$ activation (min)	$T_{½}$ cyclisation (min)
Prodrug **45**	200	100	42	
Prodrug **43**	200	100	4	47
Prodrug **22**	200	100	7.5	

Example 34

Cytotoxicity.

[0071] The anti-proliferative effect of prodrugs and parent drugs was determined *in vitro* applying seven well-characterised human tumor cell lines and the microculture sulphorhodamine B (SRB) test. The anti-proliferative effects were determined and expressed as IC_{50} values, that are the (pro)drug concentrations that gave 50% inhibition when compared to control cell growth after 5 days of incubation.

Table 1.

ID_{50} values[a,b] (ng/ml) of prodrugs and parent drugs.							
Cell Line:	MCF-7	EVSA-T	WIDR	IGROV	M19	A498	H226
Prodrug **20**	242	546	627	896	302	2303	503
Prodrug **43**	60	119	117	499	96	681	62
Prodrug **22**	11	5	5	22	7	25	7
Paclitaxel	<3	<3	<3	10	<3	<3	<3
Doxorubicin	10	8	11	60	16	90	199

[a] Drug dose that inhibited cell growth by 50% compared to untreated control cultures.

[b] SRB cell viability test.

[0072] Cell lines: MCF-7; breast cancer. EVSA-T; breast cancer. WIDR; colon cancer. IGROV; ovarian cancer. M19; melanoma. A498; renal cancer. H226; non-small cell lung cancer.

Claims

1. Compounds of the formula:

$$V\text{-}(W)_k\text{-}(X)_l\text{-}A\text{-}Z$$

wherein:

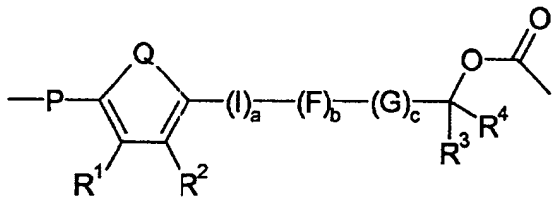
V is a specifier which is removed by an enzyme, optionally after prior binding to a receptor;
W and X are each a 1, (4+2n) electronic cascade spacer, being the same or different;
A is either a spacer group of formula $(Y)_m$, wherein
Y is a 1, (4+2n) electronic cascade spacer, or a group of formula U being a cyclisation elimination spacer;
Z is a therapeutic drug;
k, l and m are independently an integer from 0 (included) to 5 (included);
n is an integer of 0 (included) to 10 (included),

with the provisos that:

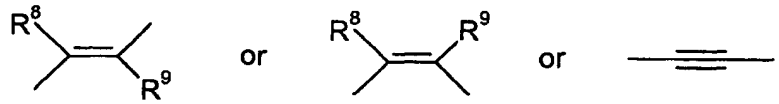
- when A is $(Y)_m$: $k+l+m \geq 1$, and
  if $k+l+m=1$, then $n > 1$;
- when A is U: $k+1 \geq 1$.

2. Compounds according to claim 1, wherein group U is an ω-amino aminocarbonyl cyclisation spacer, and Z is a drug coupled to U via its hydroxyl group.

3. Compounds of claims 1 and 2, wherein the electronic cascade spacers W, X and Y are independently selected from compounds having the formula:

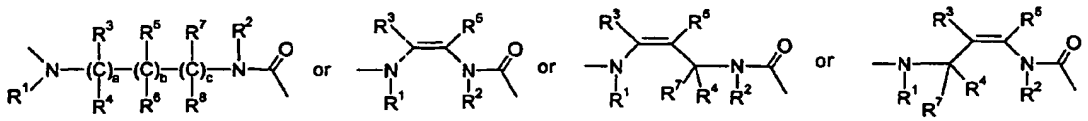


wherein
Q = $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$ P = NR^7, O, S a, b, and c are independently an integer of 0 to 5;
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, $C_{1\text{-}6}$ alkyl, $C_{3\text{-}20}$ heterocyclyl, $C_{5\text{-}20}$ aryl, $C_{1\text{-}6}$ alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x{}^1R_x{}^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic $C_{1\text{-}5}$ alkylamino, imidazolyl, $C_{1\text{-}6}$ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, $R_x{}^1$ and $R_x{}^2$ are idependently selected from a $C_{1\text{-}6}$ alkyl group, a $C_{3\text{-}20}$ heterocyclyl group or a $C_{5\text{-}20}$ aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

4. Compounds of claim 2, wherein the ω-amino aminocarbonyl cyclisation elimination spacer U is a compound having the formula:



wherein:

a is an integer of 0 or 1; and
b is an integer of 0 or 1; and
c is an integer of 0 or 1; provided that a + b + c = 2 or 3; and wherein R^1 and/or R^2 Independently represent H, C_{1-6} alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and R^3, R^4, R^5, R^6, R^7, and R^8 indepently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 can be a part of one or more aliphatic or aromatic cyclic structures, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures.

5. Compounds according to any of claims 1 to 4 wherein the specifier V contains a substrate that can be cleaved by β-glucuronidase, plasmin, prostate-specific antigen (PSA), a cathepsin, urokinase-type plasminogen activator (u-PA), or a member of the family of matrix metalloproteinases.

6. Compounds according to any of claims 1 to 4 wherein the specifier V contains a nitro-(hetero)aromatic moiety that can be removed by reduction under hypoxic conditions or by reduction by a nitroreductase.

7. Compounds according to any of claims 1 to 4 wherein the therapeutic drug Z is an anticancer agent, selected from the amino containing cytotoxic moiety daunorubicin, doxorubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, mitomycin C, mitomycin A, 9-amino camptothecin, aminopterin, actinomycin, bleomycin, N^8-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, tallysomycin, or derivatives thereof, the hydroxyl containing cytotoxic moiety etoposide, camptothecin, irinotecan, topotecan, 9-amino camptothecin, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, doxorubicin, morpholine-doxorubicin, N-(5,5-diacetoxypentyl) doxorubicin, vincristine, vinblastine, or derivatives thereof,
the sulfhydryl containing cytotoxic moiety esperamicin, 6-mercaptopurine, or derivatives thereof,
the carboxyl containing cytotoxic moiety methotrexate, camptothecin (ring-opened form of the lactone), butyric acid, retinoic acid, or derivatives thereof.

8. Compounds of any of claims 1 to 4 wherein the therapeutic drug Z represents paclitaxel or a paclitaxel derivative that is coupled to the promoiety V-$(W)_k$-$(X)_1$-U- via its 2'-hydroxyl group.

9. Compounds of any of claims 1 to 4 wherein the therapeutic drug Z is an antibiotic, an anti-inflammatory agent, or an anti-viral agent.

10. A process for the synthesis of prodrugs according to any of the claims 1 to 9 having at least one electronic cascade spacer group, and an ω-amino aminocarbonyl cyclisation elimination spacer group, connected to each other, incorporated between a specifier group and a drug molecule such that said drug molecule is connected to said cyclisation elimination spacer group, via the hydroxyl functionality of the drug molecule, by coupling a first electronic cascade spacer group, connected to said specifier group, if desired via at least one, second electronic cascade spacer group, being the same or different as said first electronic cascade spacer group, to said cyclisation elimination spacer group.

11. A process according to claim 10, wherein said drug molecule is paclitaxel, and in a first step a cyclisation elimination spacer group is coupled to paclitaxel via its 2'-hydroxyl group through a carbamate linkage via addition of the free spacer-amine to a 4-nitrophenyl carbonate activated drug, followed by deprotection to obtain a first fragment consisting of a cyclisation spacer connected with paclitaxel, and in a second step one or more 1, (4+2n) electronic cascade spacers, being the same or different, wherein n is an integer of 0 to 10, are coupled to a specifier group, subsequently activated to the corresponding 4-nitrophenyl carbonate, whereafter in a third step the fragments obtained in the first and second step are coupled to one another under basic reaction conditions at a temperature from 0 °C to room temperature.

12. Use of compounds according to any of claims 1 to 9 for the preparation of a pharmaceutical preparation for the treatment of a mammal being in need thereof.

13. Compounds according to any of claims 1 to 4 wherein the specifier V is attached to a polymer.

14. Composition comprising more than one compound according to any of the claims 1 to 4, connected with a polymeric structure.

15. A process for preparing a pharmaceutical composition containing a compound according to any of claims 1 to 4, to provide a solid or a liquid formulation for administration orally, topically or by injection.

16. Compounds according to any of claims 1 to 4, wherein the specifier V is removed by an enzyme that is transported to the vicinity of target cells or target tissue via ADEPT, PDEPT, VDEPT, or GDEPT.

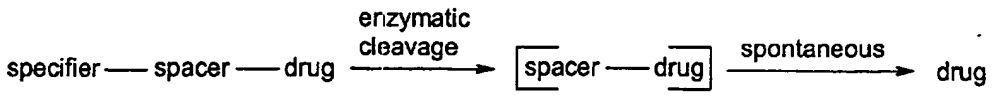


FIG. 1

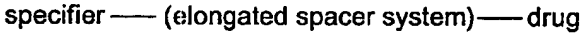


FIG. 2

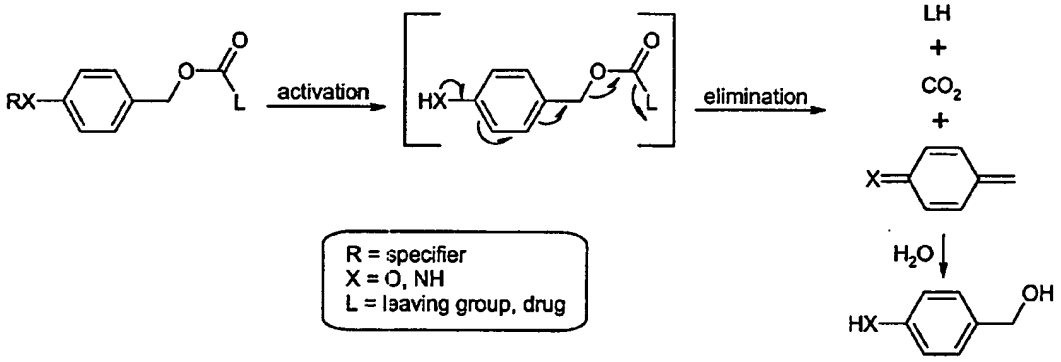


FIG. 3

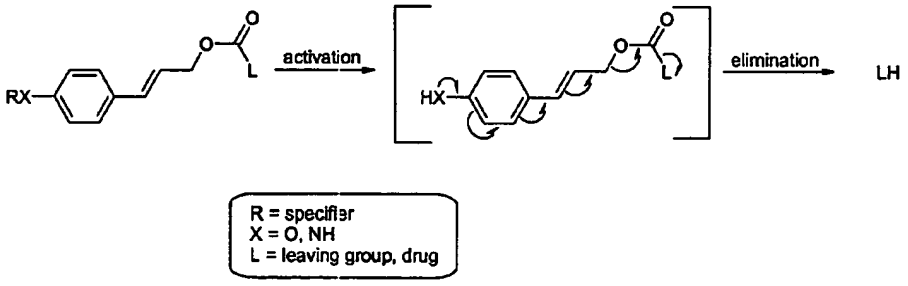


FIG. 4

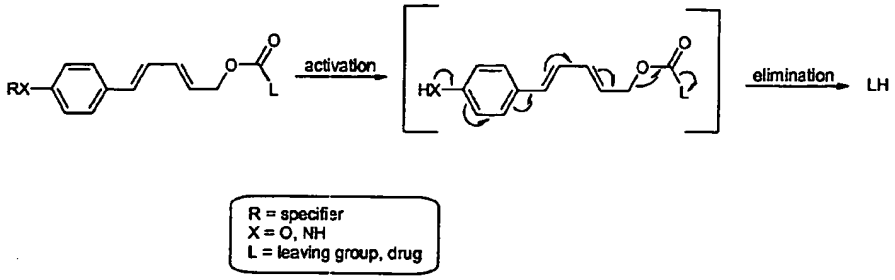


FIG. 5

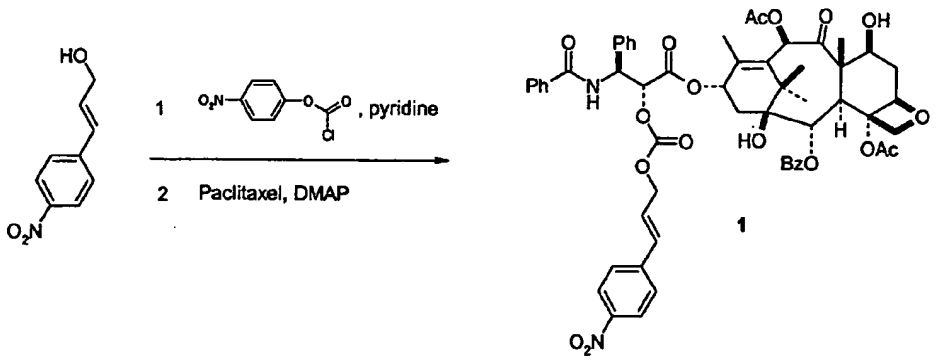


FIG. 6

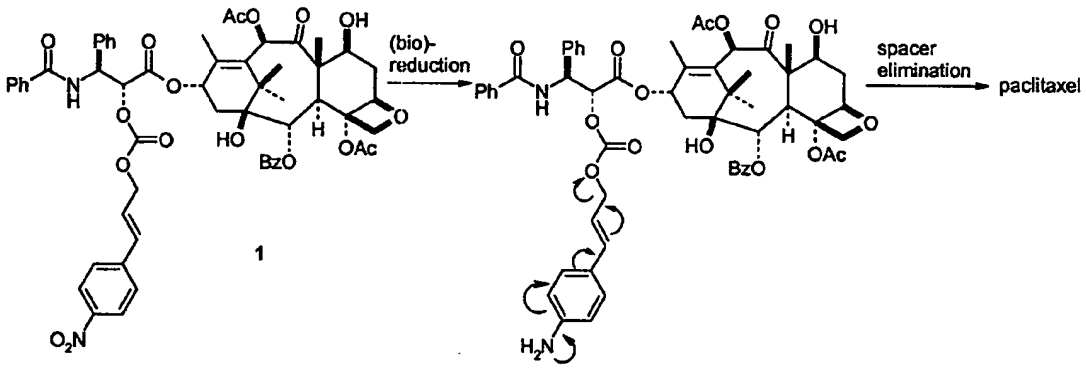


FIG. 7

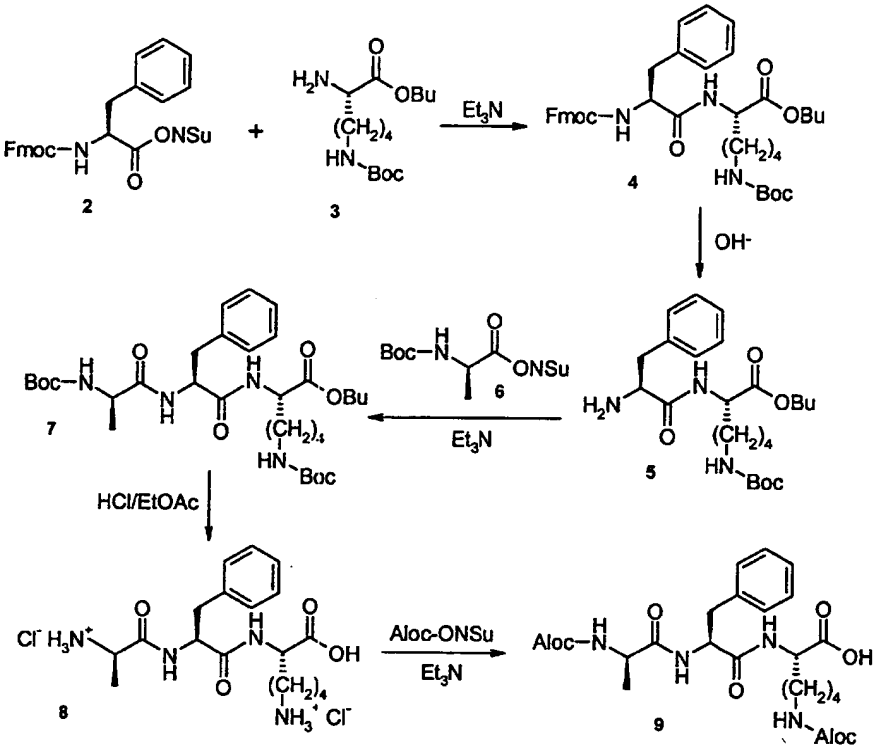

Fmoc–N
H
ONSu
O
2
+
H2N
OBu
(CH2)4
HN
Boc
3
Et3N
4
OH-
5
Boc–N
H
ONSu
6
Et3N
7
HCl/EtOAc
8
Cl- H3N+
NH3+ Cl-
Aloc-ONSu
Et3N
Aloc–N
HN
Aloc
9
OH

FIG. 8

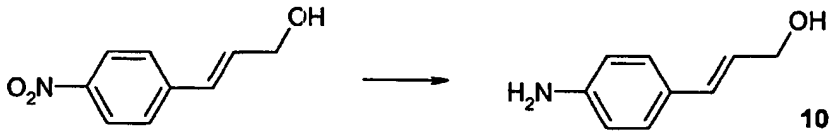

O2N
OH
H2N
10

FIG. 9

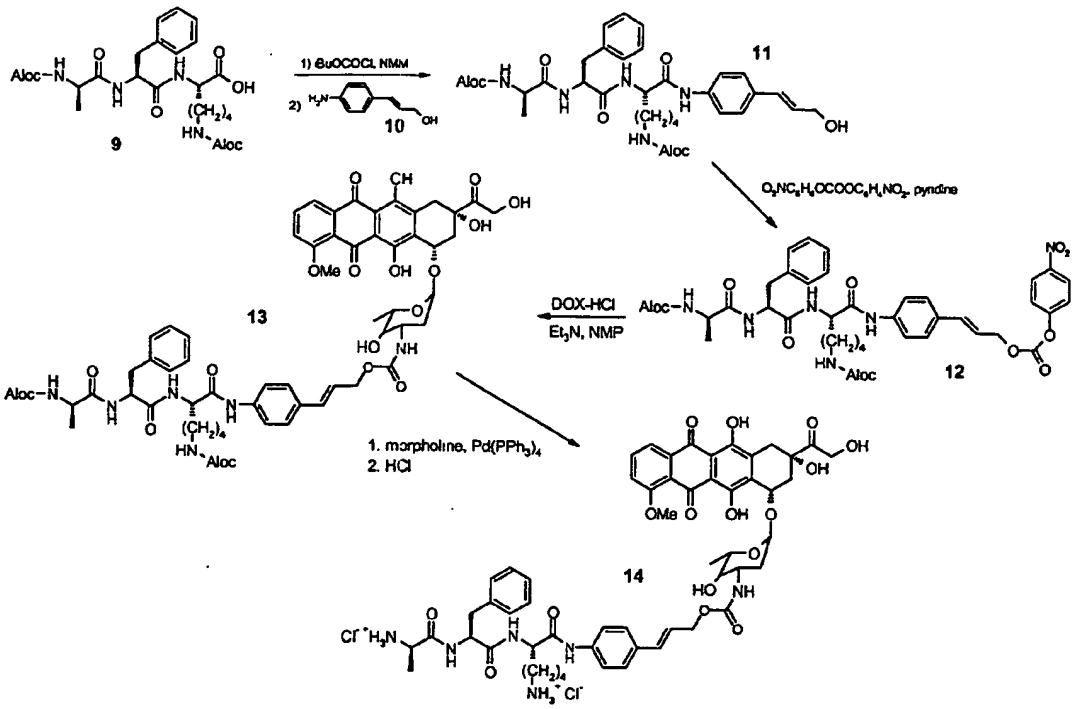


FIG. 10

specifier —— (electronic cascade spacer) —— (electronic cascade spacer)$_n$ —— drug

FIG. 11

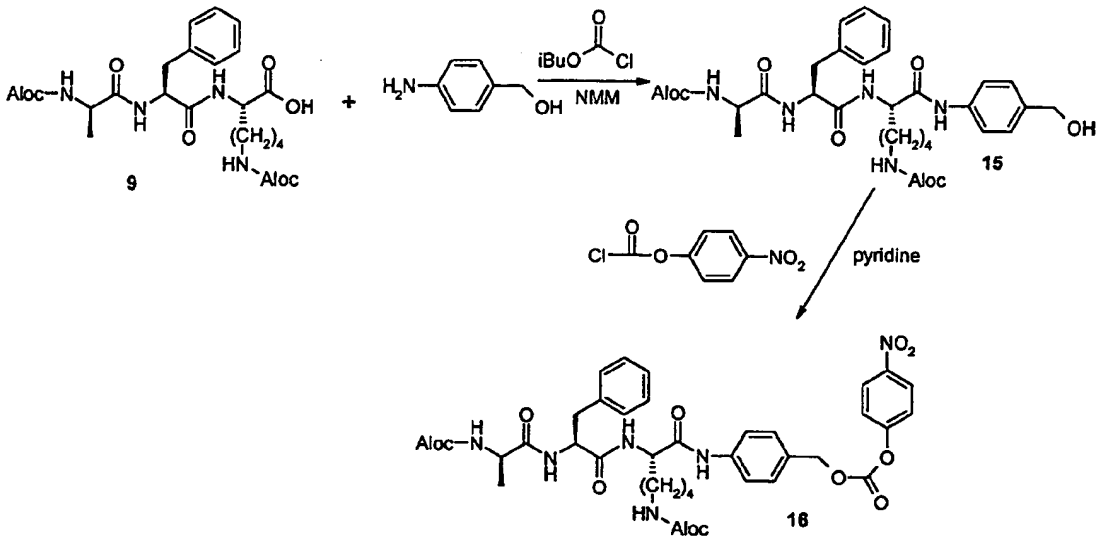


FIG. 12

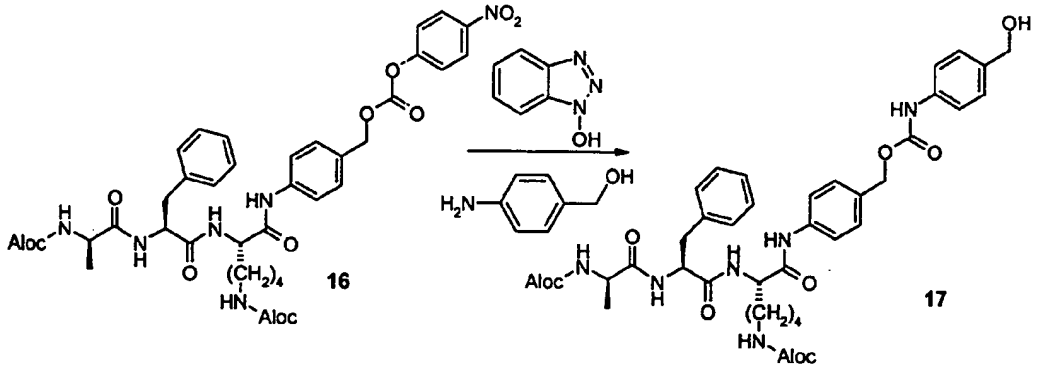


FIG. 13

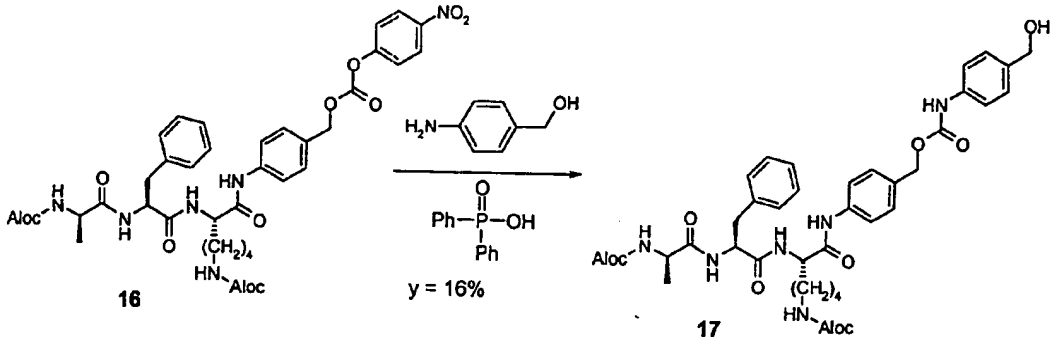

16
y = 16%
17

FIG. 14

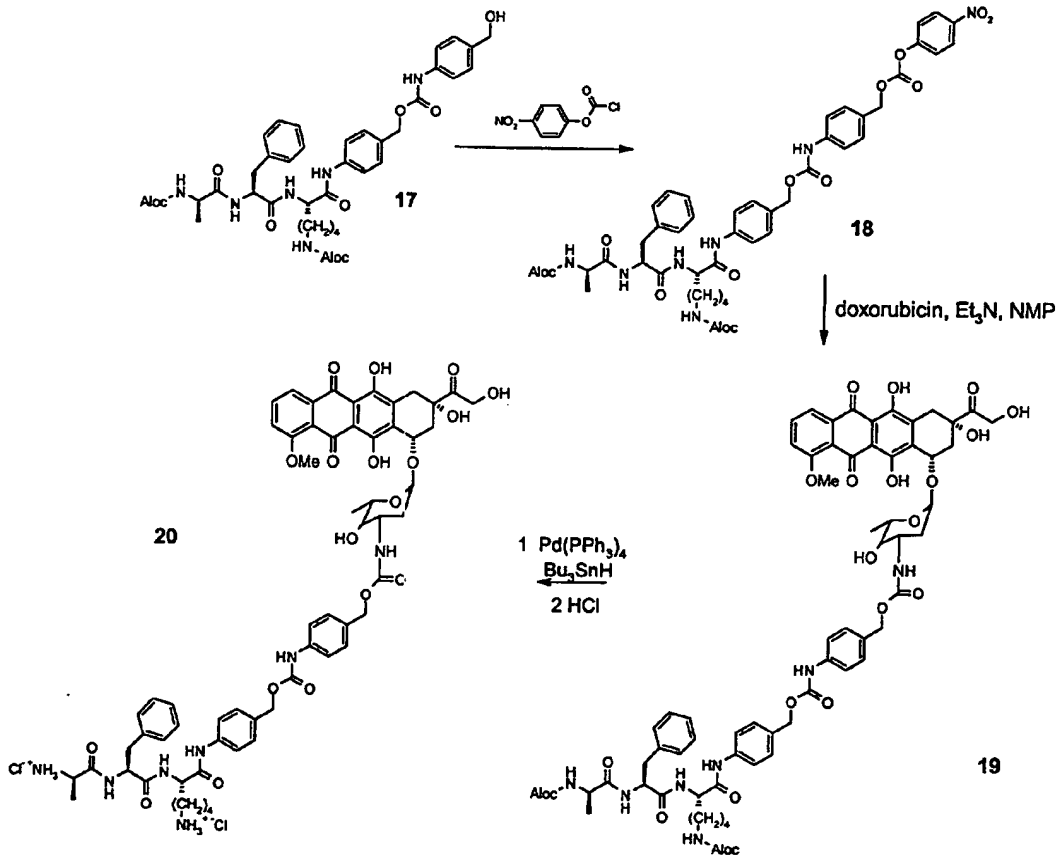

17
18
doxorubicin, Et3N, NMP
20
1 Pd(PPh3)4
Bu3SnH
2 HCl
19

FIG. 15

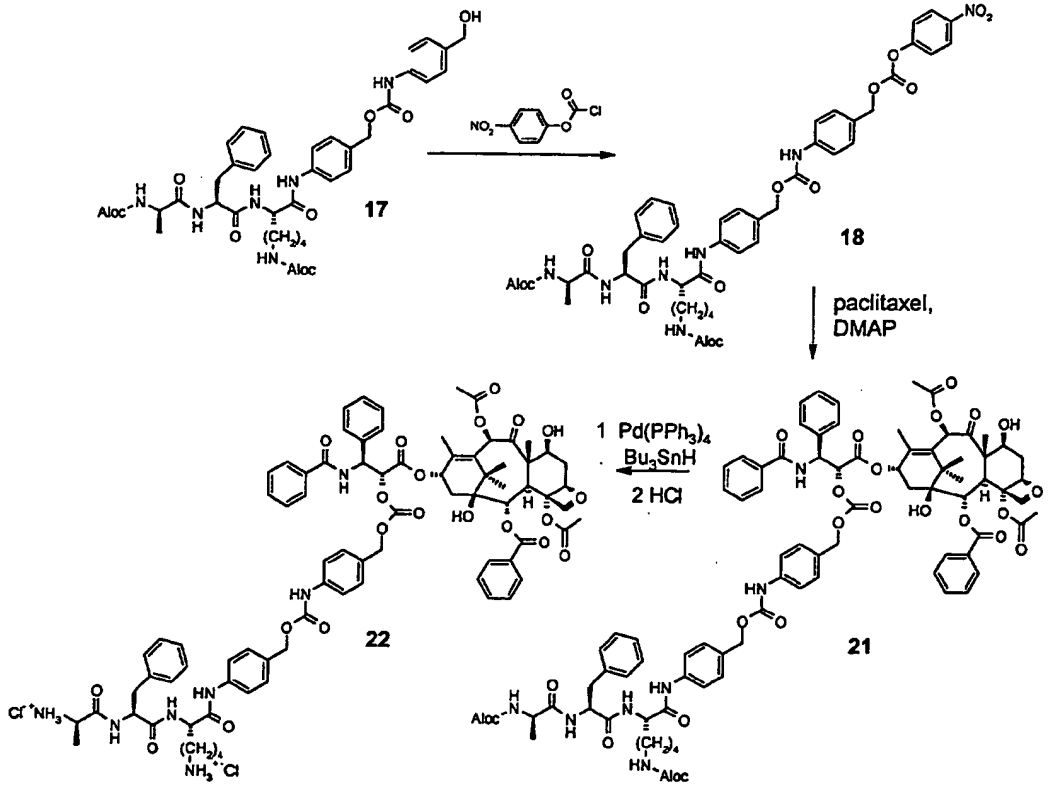


FIG. 16

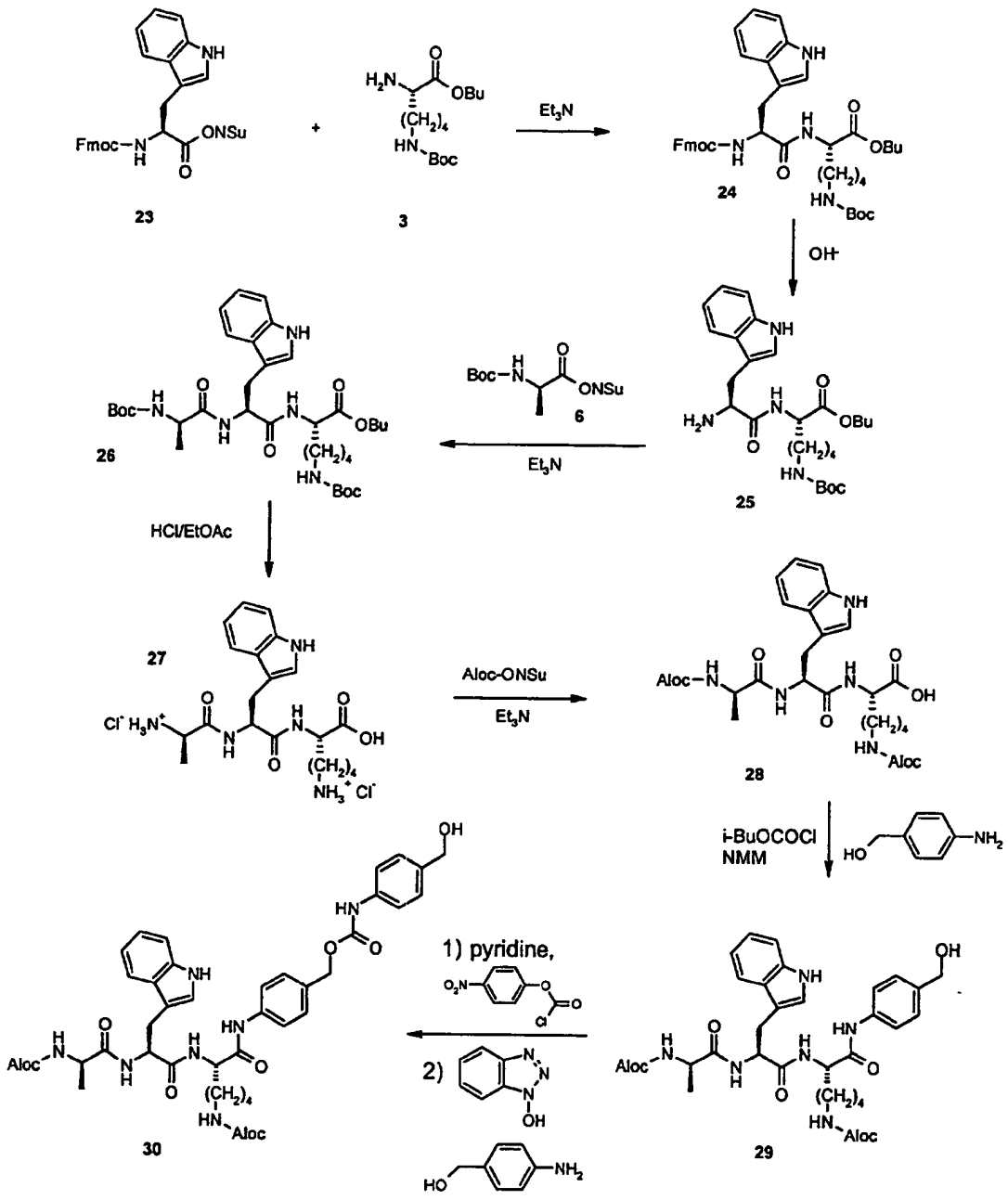

Et3N
OH-
Et3N
HCl/EtOAc
Aloc-ONSu
Et3N
i-BuOCOCl
NMM
1) pyridine,
2)

FIG. 17

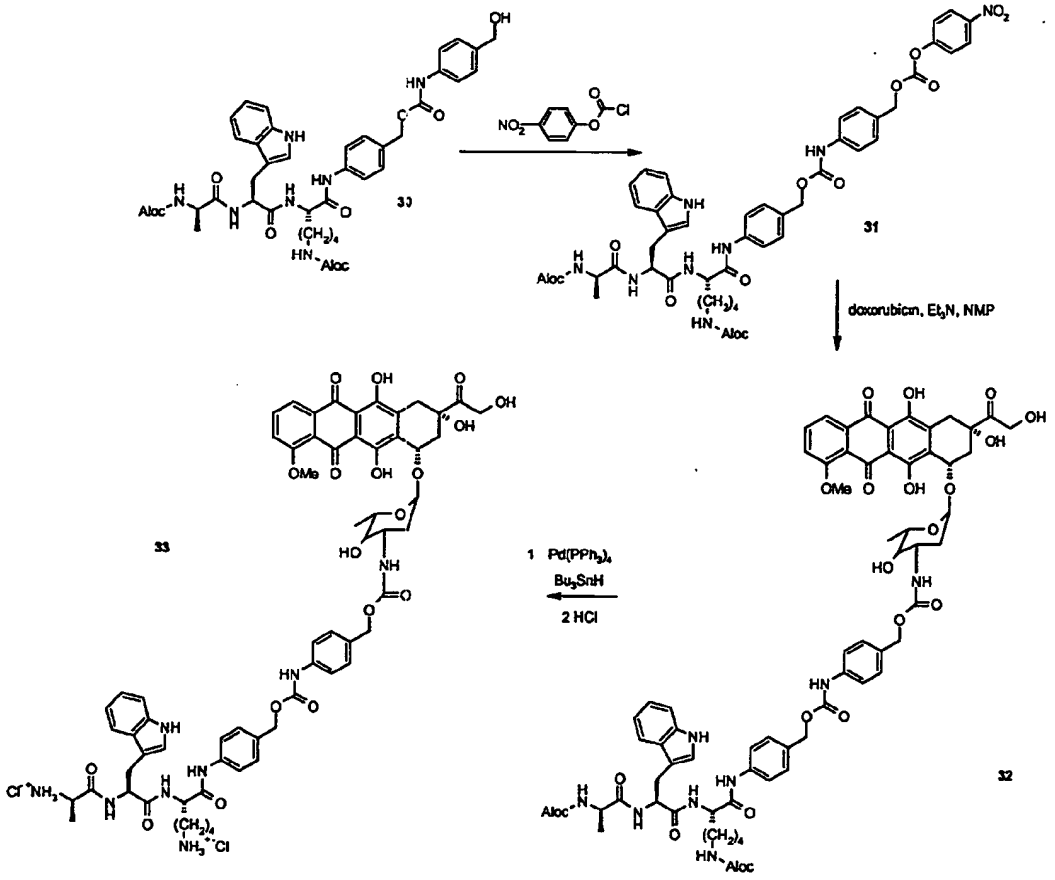


FIG. 18

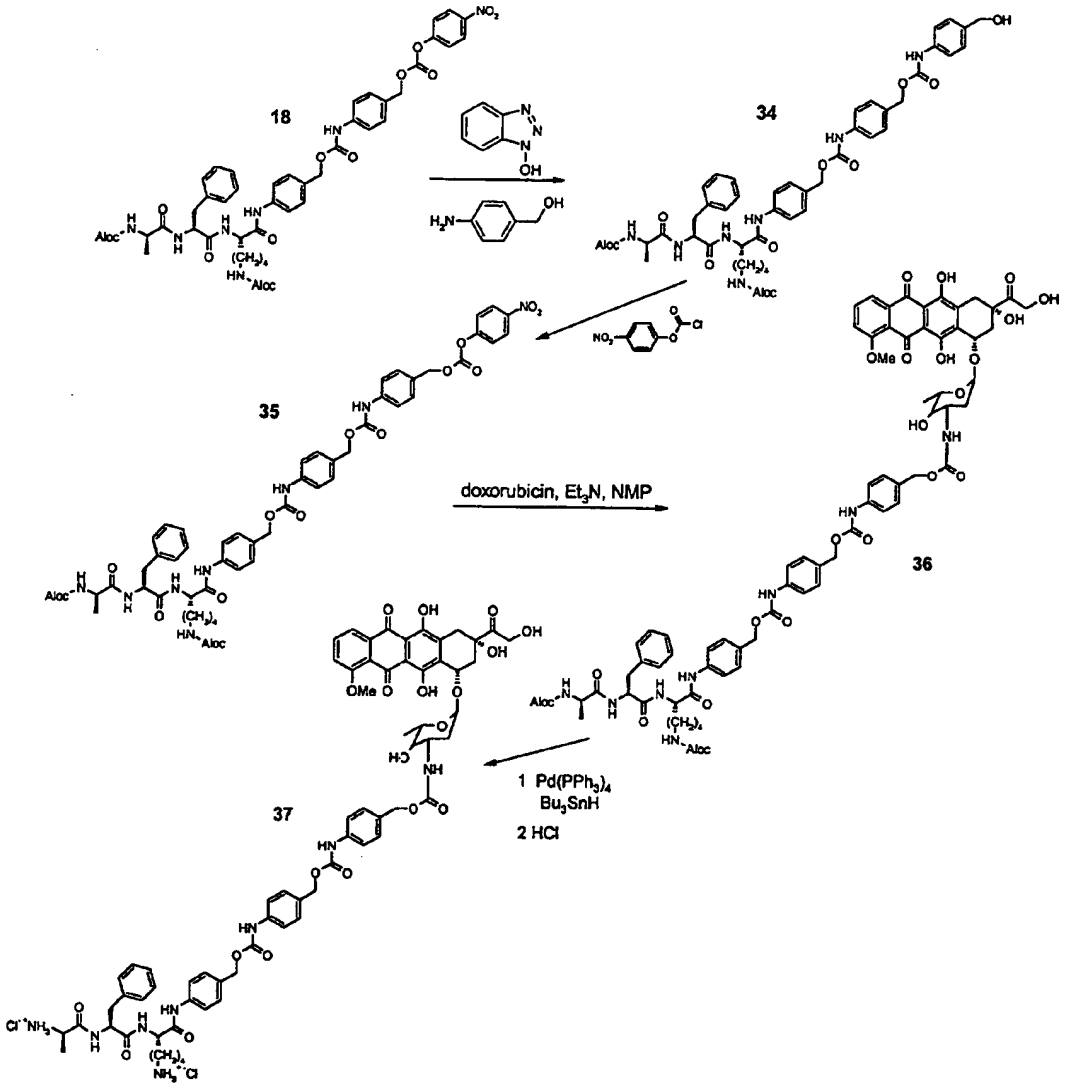


FIG. 19

specifier——(1,(4 + 2n)-electronic cascade spacer)——(cyclisation spacer)——2'-O-paclitaxel

FIG. 20

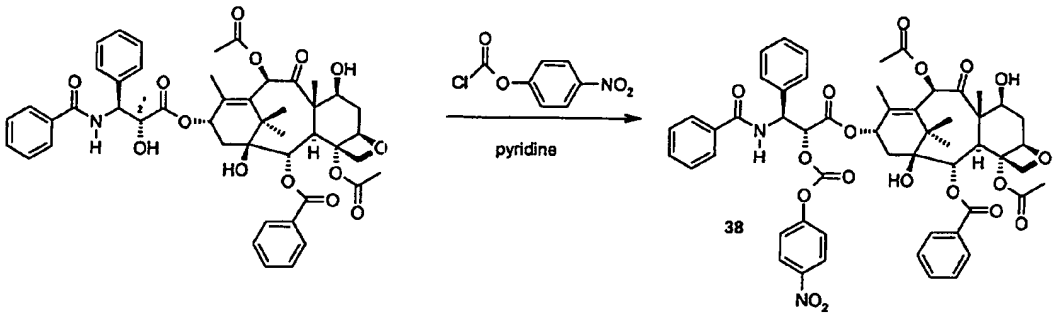


FIG. 21

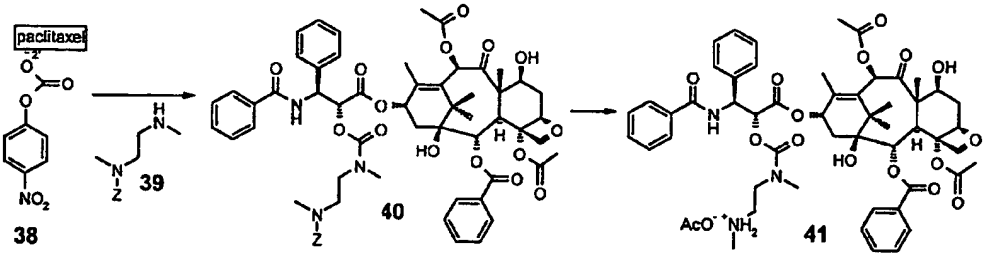


FIG. 22

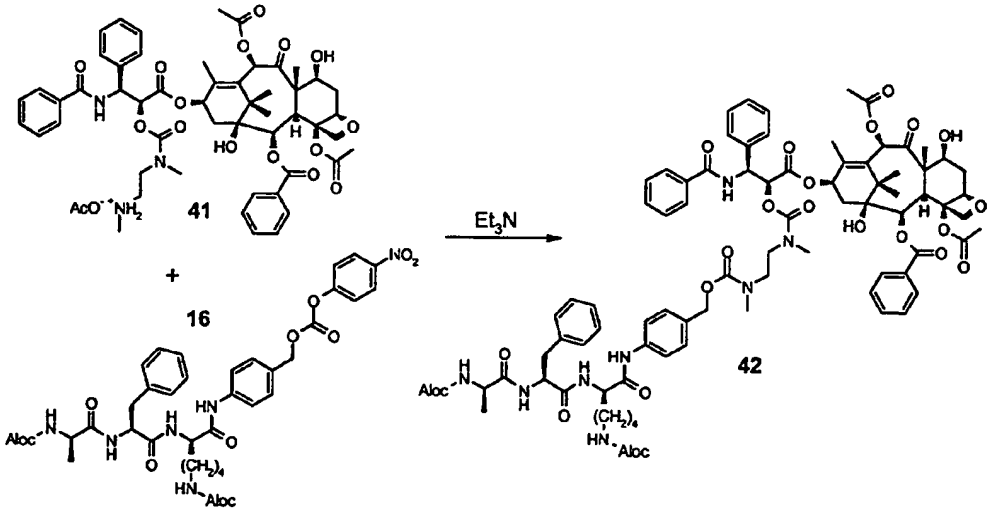


FIG. 23

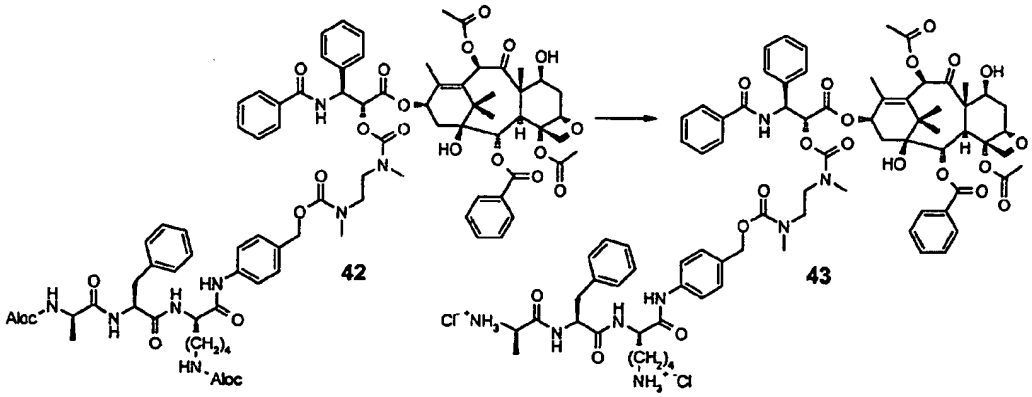


FIG. 24

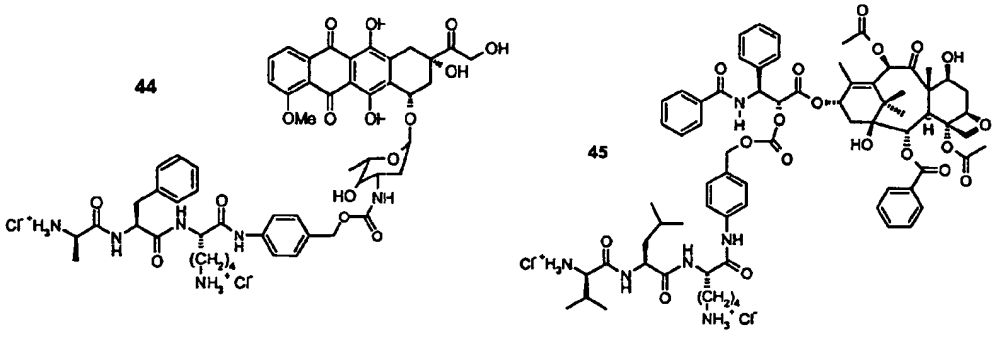


FIG. 25

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 01 20 1095

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	DE GROOT, FRANCISCUS M. H. ET AL: "Synthesis and Biological Evaluation of Novel Prodrugs of Anthracyclines for Selective Activation by the Tumor-Associated Protease Plasmin" J. MED. CHEM. (1999), 42(25), 5277-5283, XP002168683 Scheme 1,2,3 * figure 1; examples; table 1 * ---	1-16	A61K47/48
X	WO 81 01145 A (UNIV ILLINOIS) 30 April 1981 (1981-04-30)	1-16	
Y	* page 19, line 15 - line 17; claims 1,18 * * page 22 * ---	1-16	
	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	31 July 2001	Berte, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

INCOMPLETE SEARCH
SHEET C

Application Number
EP 01 20 1095

Reason for the limitation of the search:

Present claims 1-16 relate to an extremely large number of possible compounds or methods. In fact, the claims contain so many variables and possible permutations that a lack of clarity (and conciseness) within the meaning of Article 84 EPC arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely those compounds or methods recited in the examples and closely related homologous compounds.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 20 1095

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DUBOWCHIK G M ET AL: "Cathepsin B-sensitive dipeptide prodrugs. 1. A model study of structural requirements for efficient release of doxorubicin" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,OXFORD,GB, vol. 8, no. 23, 1 December 1998 (1998-12-01), pages 3341-3346, XP004143755 ISSN: 0960-894X scheme 2 , compound 26 * table 1 * ---	1-16	
A	US 5 691 371 A (DENNY WILLIAM ALEXANDER ET AL) 25 November 1997 (1997-11-25) * claims; figure 1 * ---		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	NAKAJIMA N ET AL: "Synthesis and Morphological Reversion Activity on srcNRK Cells of Pyrimidinylpropanamide Antibiotics, Sparsomycin, Sparoxomycin A1, A2, and Their Analogues" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,GB,OXFORD, vol. 8, no. 23, 1 December 1998 (1998-12-01), pages 3331-3334, XP004143753 ISSN: 0960-894X scheme 1,4 --- -/--	1-16	

EPO FORM 1503 03.82 (P04C10)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 01 20 1095

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	DUBOWCHIK, GENE M. ET AL: "Cathepsin B-sensitive dipeptide prodrugs. 2. Models of anticancer drugs paclitaxel (taxol), mitomycin C and doxorubicin" BIOORG. MED. CHEM. LETT. (1998), 8(23), 3347-3352, XP002168684 scheme 1 * figure 1; table 1 *	1-16	
Y	WO 98 13059 A (SQUIBB BRISTOL MYERS CO) 2 April 1998 (1998-04-02)	1-16	
X	* claim 1 *	1	
X	EP 0 624 377 A (SQUIBB BRISTOL MYERS CO) 17 November 1994 (1994-11-17) formula 3 * claims; examples *	1-16	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	CHAKRAVARTY, PRASUN K. ET AL: "Plasmin-activated prodrugs for cancer chemotherapy. 2. Synthesis and biological activity of peptidyl derivatives of doxorubicin" J. MED. CHEM. (1983), 26(5), 638-44, XP002168690 scheme 1,2	1-16	

EPO FORM 1503 03.82 (P04C10)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 20 1095

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

31-07-2001

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		EP 0038357 A	28-10-1981
US 5691371 A	25-11-1997	AU 6859394 A	20-12-1994
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		JP 8510469 T	05-11-1996
		WO 9427954 A	08-12-1994
		US 5872129 A	16-02-1999
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		EP 0961619 A	08-12-1999
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		AU 6302694 A	17-11-1994
		CA 2123363 A	15-11-1994
		CN 1100426 A	22-03-1995
		CZ 9401187 A	16-11-1994
		FI 942237 A	15-11-1994
		HU 66485 A	28-11-1994
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